

# The influence of different winemaking techniques on the extraction of grape tannins

By

**Anton Pieter Nel**



Thesis presented in partial fulfillment of the requirements for the degree of  
**Master of Agricultural Science**

At  
**Stellenbosch University**  
Department of Viticulture and Oenology, Faculty of AgriSciences

*Supervisor:* Prof Marius Lambrechts  
*Co-supervisor:* Prof Pierre van Rensburg

December 2010

## Declaration

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Date: 28 February 2011

## Summary

Grape and wine phenols consist of flavanols which is the building blocks for tannins. These building blocks are called monomers which consist of catechins, epicatechins, epigallocatechins and epicatechin-gallate. Tannin is important in wine as it contributes to bitterness, mouth feel (astringency) and maturation potential of the wine. Furthermore it has a health benefit as an antioxidant. Anthocyanins are responsible for the colour of red wine. The anthocyanins combine with tannins to form stable polymeric pigments. Due to the importance of tannins and anthocyanins in wine, it is imperative that different winemaking techniques are used to extract as much of these components as possible and that the analysis is done quickly and accurately.

The aim of this study was to evaluate different winemaking techniques and their extraction of tannins and anthocyanins into the wine. Too much tannin extraction can have a negative effect on the sensory quality of the wine. Therefore a second aim was to evaluate the mouth feel properties of a Shiraz wine. A third aim was to compare the two tannin precipitation methods in terms of time efficiency, repeatability and the ease of practice.

To investigate the amount of tannin concentration extracted by different winemaking techniques, two cultivars (Cabernet Sauvignon and Shiraz) were used. These treatments included the addition of an enzyme during fermentation [E], cold maceration [CM], post maceration [PM] and the combination of cold and post maceration [CM+PM]. The grapes were harvested in two different climatic areas during the 2008 and 2009 vintages. The two climatic areas were classified according to the Winkler scale as a III (Morgenster) and a IV (Plaisir de Merle). The grapes were harvested at two different ripeness levels in order to evaluate the effect of the different winemaking processes on the extraction of tannins and anthocyanins. One harvest was before (LB) and the other after (HB) the commercial harvest.

The results of this study showed significant differences in the phenolic composition of the wines. It was found that the warmer area showed higher tannin concentrations than the cooler area for both cultivars. In the 2008 Cabernet Sauvignon the CM extracted higher concentrations of tannin from the cooler area at both ripeness levels. In the warmer area, CM extracted the highest tannin concentration HB, but the CM+PM

extracted the highest tannin concentration from Cabernet Sauvignon at the LB and CM at the HB of the warmer area. In 2009 the PM extracted the highest concentration of tannin at the lower ripeness level, while the E treatment extracted the highest concentration from the warmer area. In the cooler area the CM+PM extracted the highest concentration of tannin at a lower ripeness level, while there were no significant differences between the different treatments at the higher ripeness level. The highest anthocyanin concentration was found in the cooler area. The CM treatment was found to have no effect on anthocyanin extraction.

Different methods are available to quantify the tannin concentration in wine. Two of the most popular tannin analytical methods are the bovine serum albumin (BSA) and the methyl cellulose precipitable tannin (MCP) methods. The BSA method is a very complex method which uses at least 3 times more reagents than the MCP method. The MCP method only analyzes tannins, while the BSA method analyzes tannins, monomeric pigments (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP).

In this study a good correlation was found between the two tannin precipitation methods ( $R^2 = 0.88$ ). There is controversy regarding the variability of these methods. Some scientists found that the two methods show a good correlation with HPLC, while others found that there was no such correlation between the precipitation methods and the HPLC. The MCP method had a practical advantage as it could be performed in half the time required for the BSA method. This has a significant impact in scenarios where a high sample throughput is required although it only measures total tannin.

The phenolic composition and mouth feel of the wine was strongly influenced by the climatic area. In the warmer area the effect of tannin concentration on mouth feel was much less than in the cooler area. The wine made of riper grapes, was more grippy, bitter and numbing than the wines made from greener grapes. The E treatment was especially associated with a dry, grippy sensation.

## Opsomming

Druif en wyn fenole bestaan uit flavanole wat weer die boublokke is van tanniene. Hierdie boublokke, wat bekend staan as monomere, bestaan uit katesjiene, epikatesjiene, epigallokatesjiene en epikatesjien-gallaat. Tanniene is belangrik in wyn aangesien dit bydra tot bitterheid, mondgevoel (vrankheid) asook die verouderingspotensiaal van wyn. As antioksidante hou dit ook gesondheidsvoordele in. Antosianiene dra by tot die kleur van rooiwyn. Antosianiene kombineer met tanniene om meer stabiele polimeriese pigmente te vorm. As gevolg van die belangrikheid van tanniene en antosianiene is dit van uiterse belang dat verskillende wynmaak tegnieke gebruik word om ekstraksie in die wyn te bevoordeel en dat die analitiese metode so vinnig en akkuraat as moontlik gedoen word.

Die eerste doel van hierdie studie was om die ekstraksie van tanniene en antosianiene deur middel van verskillende wynmaak tegnieke te evalueer. Te veel tanniene in die wyn kan negatiewe sensoriese kwaliteit tot gevolg het. Daarom is die tweede doel om die sensoriese kwaliteit van Shiraz wyn te evalueer. Die derde doel van hierdie studie was die twee tannien presipitasie metodes met mekaar te vergelyk in terme van die moeilikheidsgraad van die metode, tyd doeltreffendheid en herhaalbaarheid.

Verskillende wynmaak tegnieke (ensiem byvoegings [E], koue maserasie [CM], verlengde dopkontak [PM] en 'n kombinasie van koue maserasie en verlengde dopkontak [CM+PM]) is vergelyk ten opsigte van tannien en antosianien ekstraksie. In 2008 en 2009 is twee kultivars (Cabernet Sauvignon en Shiraz) in twee verskillende klimatologiese areas gepars. Hierdie areas is geklassifiseer in die Winklerskaal as 'n IV (Plaisir de Merle) en 'n III (Morgenster). Om die effek van die verskillende wynmaak tegnieke op die ekstraksie van antosianiene en tanniene te vergelyk, is hierdie twee kultivars by twee verskillende rypheidsgrade geoes. Die eerste oes was net voor kommersiële oes (LB) en die tweede oes het net na kommersiële oes (HB) plaasgevind. Die 2009 Shiraz wyn is organolepties beoordeel om die effek van die verskillende wynmaak tegnieke op die wyn se mondgevoel te vergelyk.

Die resultate van hierdie studie toon beduidende verskille in die fenoliese samestelling van die wyne. Dit is gevind dat die warmer area hoër tannien konsentrasies het as die koeler area. In 2008 het die CM+PM die meeste tanniene uit die Cabernet Sauvignon

geëkstraheer by LB en die CM by HB in die warmer area. Die CM het in die koeler area meer tanniene geëkstraheer by beide die LB en HB rypheidsgrade. In 2009 het PM die meeste tanniene geëkstraheer by LB terwyl E die meeste tanniene geëkstraheer in die warmer area. In die koeler area het CM+PM die meeste tanniene geëkstraheer, terwyl geen van die behandelings 'n effek gehad het by HB. Die meeste antosianien konsentrasie was in die koeler area gevind as in die warmer area. In beide 2008 (LB en HB) en 2009 (LB) het CM die meeste antosianiene geëkstraheer, terwyl geen behandeling 'n effek gehad het by HB.

Twee van die mees populêre tannien analitiese metodes is die BSA (bovine serum albumien) en die MCP (metielsellulose presipitasie) metodes. Die BSA metode is 'n baie meer ingewikkelde metode waarvoor drie keer meer reagense gebruik word as vir die MCP metode. Maar waar die MCP net tanniene ontleed, ontleed die BSA metode tanniene, monomere (MP), klein polimeriese pigmente (SPP) en groot polimeriese pigmente (LPP). Dit help indien daar gekyk wil word na die evolusie van polimeriese pigmente.

In hierdie studie is bevind dat daar 'n redelike korrelasie ( $R^2 = 0.88$ ) tussen die BSA en MCP metode bestaan. Die herhaalbaarheid van die metodes het redelike kontroversie veroorsaak, waar sommige navorsers bevind het dat die BSA metode nie so herhaalbaar is soos eers bevind is nie. Die MCP metode het 'n praktiese voordeel aangesien dit in die helfde van die tyd van die BSA metode uitgevoer kan word. Dit het 'n groot impak indien 'n groot hoeveelheid monsters ontleed moet word.

Die fenoliese samestelling en mondgevoel word sterk beïnvloed deur die klimatologiese area. In die warmer area was die effek van tannien konsentrasie op mondgevoel kleiner as in die koeler area. Die wyn van ryper druiwe het meer harder, verdovingseffek en bitter nasmaak gehad as by die wyn van groener druiwe. Die ensiem behandeling was meer geassosieerd met droë mond gevoel.

This thesis is dedicated to  
Helma Nel, my wife, for all her assistance, love and dedication.

Hierdie tesis is opgedra aan my vrou, Helma Nel, vir haar volgehoue liefde en  
ondersteuning.

## Biographical sketch

Anton Pieter Nel was born in Windhoek, Namibia (the old South West Africa) in 1969. He matriculated at Adamantia High School, Kimberley in 1987. After matric he completed two years of military service in 1 Parachute Batalion. Anton chooses a career in the wine industry due to his love for science and nature. During his 14 years in the industry he worked for KWV (1990), was an assistant winemaker at Uitvlugt Montagu (1994) and Louwshoek-Voorsorg (1996) before becoming a winemaker at Kango Wine Cellar (2001) in Oudtshoorn. He is currently employed by Distell.

He obtained a BScAgric-degree at the University of Stellenbosch in 2007, majoring in Enology and Viticulture. He enrolled for the MScAgric-degree in Enology during 2008 at the same institution. Anton is married to Helma Nel, and father of two children, Pieter and Hesmarie Nel.

His philosophy in life is "*in vino veritas*": "in wine the knowledge"

## Acknowledgements

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

- Our **Heavenly Father** for keeping His hand over me and my family in the course of my studies.
- **Prof Marius Lambrechts** who acted as supervisor to this project. For his critical reading of this manuscript and for his continuous input and advice.
- **Prof Pierre van Rensburg** who acted as co-supervisor. For his critical reading of this manuscript and also for his continuous input and advice.
- **Leannie Louw** and the Sensory Analysis group of Distell as well as the panelists.
- **Winetech** for their funding of my project.
- **Prof Martin Kidd** as the consulting statician for this project. For his advice and patience and for always making time to accommodate the processing of data involved in the study.
- **MP Botes** and the cellar workers at the experimental cellar at Adam Tas, namely **Clement** and **Mark**, for helping with the pressing, filtering and bottling of the wines.
- To my wife, **Helma**, my son, **Pieter**, and my newborn baby girl, **Hesmarie**, for their support and patience.

# Preface

This thesis is presented as a compilation of chapters. Each chapter is introduced separately and is written according to the style of the *South African Journal of Enology and Viticulture*.

**Chapter 1**      **GENERAL INTRODUCTION AND PROJECT AIMS**

**Chapter 2**      **LITERATURE REVIEW**

Tannins and anthocyanins: from origin to wine

**Chapter 3**      **RESEARCH RESULTS**

The influence of different winemaking techniques on the extraction of grape tannins from Cabernet Sauvignon and Shiraz grapes.

**Chapter 4**      **RESEARCH RESULTS**

The influences of different winemaking techniques on the mouth feel of Shiraz grapes.

**Chapter 5**      **GENERAL DISCUSSION AND CONCLUSIONS**

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# **Chapter 1**

## **Introduction and project aims**

# 1. GENERAL INTRODUCTION AND PROJECT AIMS

## 1.1 INTRODUCTION

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The accumulation of flavonoids in a ripening grape berry occurs in two stages, namely the accumulation of proanthocyanidins before veraison and the accumulation of anthocyanins after veraison (Bogs *et al.*, 2005 & 2007). Although the genes for the proanthocyanidins and anthocyanins already exist at flowering, the genes for anthocyanins are only expressed at the onset of veraison (Bogs *et al.*, 2007).

The proanthocyanidins are synthesized through the flavonoid biosynthetic pathway via the shikimate (Marques *et al.*, 2007) and phenylpropanoid pathways (Ferrer *et al.*, 2008). The synthesized proanthocyanidins are then transported to different sinks of the grape berry, such as the skin and seeds. The development of the proanthocyanidins in the skins and seeds differ in their polymeric length. For instance the mDP of the skins range from 25-40 subunits (Downey *et al.*, 2003; Kennedy *et al.*, 2000), while the mDP of the seeds are 4-6 subunits in length (Downey *et al.*, 2003). The flavan-3-ols composition of the skins and seeds also differ. Both the skins and seeds contain (+)-catechin, (-)-epicatechin and (-)-epigallocatechin, but only the seeds contain (-)-epicatechin-gallate (Kennedy *et al.*, 2000).

Anthocyanins only start to accumulate at the onset of veraison. Anthocyanins accumulate in the vacuoles of the epidermic cells of the berry skins (Ortega-Regules *et al.*, 2006). When anthocyanidins glycosylate with glucose anthocyanins are formed (Castaneda-Ovando *et al.*, 2009). There are five basic anthocyanins that occur in red grapes, namely: cyanidin, delphinidin, peonidin, petunidin and malvidin (Liang *et al.*, 2008). These five anthocyanins vary in hue from pink to purple-blue (Castaneda-Ovando *et al.*, 2009). The colour depends on the hydroxyl groups on the B-ring of the flavylium cation. These five anthocyanins can also be acylated with acetate and coumaric acid to give ultimately fifteen different colour forms (Gomez-Plaza *et al.*, 2008).

Together these flavonoids (proanthocyanidins and anthocyanins) have a very important sensory impact on wine and the subsequent wine quality. Tannins, for instance, enhance the mouth feel (Noble, 1994 and Gawel, 1998) of the wine. The mouth feel of

wine is so complex that Gawel *et al.* (2000) designed a mouth feel wheel to help tasters in defining the different mouth feel descriptors. Mouth feel can be roughly divided in two sensory perceptions. Bitterness is a taste sensation which can be detected at the back of the tongue (Gawel, 1998). Astringency is a tactile sensation which can normally be detected after the wine was expectorated (Gawel, 1998). Anthocyanins, on the other hand, are responsible for the colour of wine. A combination of tannins and anthocyanins, in a 1:4 ratio, has a stabilizing effect on the colour (Monagas *et al.*, 2005) and which will improve the maturation potential of the wines (Lorenzo *et al.*, 2005).

Several methods are available to the farmer/viticulturist to establish the quality of grapes. Methods like the traditional °Brix, pH and TA, °Brix:pH, TA:pH, °Brix:TA or °Brix x (pH)<sup>2</sup> (du Plessis and van Rooyen, 1982) can be used, but they have all limited success. Another method that is used by farmers/viticulturists is the tasting of berries in the vineyards. The colour of the pips is an indication of berry ripeness as the colour of the pips varies as the grapes ripen. Finally, the colour of his spit is an indication of the amount of anthocyanins that have been extracted; anthocyanin extraction increases during ripening. Chewing ripe berries with high levels of extracted anthocyanins will result in a purple colour change in ones spit. This principle was used by Glories (1984b) in his analysis for phenolic ripeness.

Glories (1984a) also found that there were a correlation between total anthocyanin (at pH1) and extractable anthocyanin (at wine pH of 3.2). In green berries the difference between total and extractable anthocyanins is very big, but as the berry ripens this difference become smaller. Therefore the difference of total anthocyanin and extractable anthocyanin (expressed as a percentage of total anthocyanin) are used to predict phenolic ripeness. Furthermore, Ortega-Regules *et al.* (2006) defines phenolic maturity as the time when the concentration of grape anthocyanins is at its maximal. Although these are not foolproof methods, they certainly give indications to the farmer/viticulturist as to the potential quality of the grapes and, of course, the ripeness.

Anthocyanins are water soluble and are therefore more easily extracted from grape skins before fermentation (Castaneda-Ovando *et al.*, 2009). As the grapes are inoculated after destemming, ethanol is produced which extracts more of the phenolic compounds (Sacchi *et al.* 2005). These phenolic compounds are more soluble in an alcohol solution than in a water solution. Red wine ferments at a higher temperature and

therefore at a faster tempo than white wine. Fermentation of red wine typically takes about a week to finish (personal experience). After fermentation the wine are pressed and the wine is left for malolactic fermentation (MLF). So in effect, the wine has about a week to extract as much anthocyanin and phenolic compounds out of the grape skins and seeds, which is not always enough. The amount of extraction that can take place during this time can be influenced by the cultivar and ripeness level of the grapes that is used for winemaking. Some grape cultivars have few anthocyanins (Pinot noir) while others gave deep coloured wine (Pinotage) and a few are known as tenturier grapes where anthocyanin are present in the skins as well as the flesh of the grape.

There are different methods available to the winemaker for enhancing the extraction of tannin and anthocyanins from the grape berry. These methods vary from a premaceration (cold soaking) method where the grapes mulch is cooled down to about 10°C for at least three days (Gomez-Plaza *et al.*, 2000) prior to fermentation. This is done to extract the anthocyanins from the berry skins. With the post maceration or extended maceration, the wine is left on the skins for a further two weeks after fermentation so that the alcohol in the wine can extract more tannin from the skins and seeds (Joscelyne and Ford, 2008). With thermovinification the grape mulch is heated to 60-80°C for 20-30 minutes (Ribereau-Gayon *et al.*, 2000). During this time the cell walls are ruptured and the tannins and anthocyanins are extracted. Whole bunch fermentation (carbonic maceration) can also be used, but fruitier aroma compounds are extracted with this method than anthocyanins (Sacchi *et al.*, 2005). Other methods that can be used are the addition of enzymes (Arnous & Meyer, 2009) and sulphur (Spagna *et al.*, 2003) which will also promote the extraction of colour and tannins.

There are different methods that can be used to quantify the concentration of tannins in a wine. Colorimetric methods (Makkar, 1989; Souquet *et al.* 1996; Sun *et al.*, 1998 & Monagas *et al.*, 2005) use a change in colour to measure the amount of tannins while gravimetric methods (Ginger-Chavez *et al.*, 1997) uses ytterbium to bind to the tannins and settle by gravitation. The most popular methods nowadays are the precipitation methods. In these methods a polysaccharide, methyl cellulose – MCP (Sarneckis *et al.*, 2006), or a protein, bovine serum albumin – BSA (Hagerman & Butler, 1978) which was later modified by Habertson, (2003) are used to precipitate the tannin. These methods are used with varying results, as it was found that the BSA method were not consistent in its results (Habertson and Downey, 2009). High performance liquid chromatography

(HPLC) is the best method for the quantification of tannins and anthocyanins. However, a poor correlation was found between the results from the BSA method and HPLC (Seddon and Downey, 2008).

## 1.2 PROJECT AIMS

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As mentioned, tannin is very important to wine as it contribute to taste (bitterness) and mouth feel (astringency) of the wine, also it contribute to the maturation potential of wine as well as health benefits. Anthocyanin contributes to the colour of the wine. Therefore the specific aims of this study were as follows:

- 1) To evaluate the phenolic ripeness of the grapes with the Glories method
- 2) To evaluate the extraction of tannin and anthocyanin by the winemaking process of cold maceration
- 3) To evaluate the extraction of tannin and anthocyanin by the winemaking process of post maceration
- 4) To evaluate the extraction of tannin and anthocyanin by the winemaking process of a combination of cold and post maceration
- 5) To evaluate the extraction of tannin and anthocyanin by using pectolytic enzymes
- 6) To evaluate the extraction of tannin concentration by using two precipitation methods, namely Bovine serum albumin (BSA) and methylcellulose precipitable (MCP) of tannin methods
- 7) To evaluate the effect of the different winemaking processes on the mouth feel of the wine

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# **Chapter 2**

## **Literature review**

**Tannins and Anthocyanins: from origin to wine**

## 2. LITERATURE REVIEW

### 2.1 INTRODUCTION

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When talking about the origin of tannins and anthocyanins in grapes and wine, the term flavonoid biosynthesis springs to mind. However, the origin of the precursors that enters the flavonoid biosynthetic pathway must first be considered. Everything starts with budbreak in the early spring (Coombe, 1995). The grapevine starts to push its leaves into the open and the chlorophyll in the leaves use sunlight to start photosynthesis. From photosynthesis the NADP<sup>+</sup> molecules are used in the Calvin cycle (Jackson, 1994) to produce erythrose-4-phosphate (Marques *et al.*, 2007). Erythrose-4-phosphate condenses with phosphoenolpyruvate to produce phenylalanine in the phenylalanine pathway (Ferrer *et al.*, 2008). In the phenylpropanoid pathway the phenylalanine are deaminated to form chalcone. It is this chalcone that is the precursor for the flavonoid biosynthetic pathway which will synthesize anthocyanins and proanthocyanidins (Bogs *et al.*, 2007).

The word tannin is a collective name for a group of phenols that exist naturally in the grape berry. This group of phenols is further subdivided into flavonoids and non-flavonoids (Monagas *et al.*, 2005). The non-flavonoids consists of the benzoic acids and the cinnamic acids, while the flavonoids consists of flavanols, flavonols, flavan-3,4,-diols and anthocyanins (Monagas *et al.*, 2005). The basic building blocks for tannin comes from the flavanol subgroup and consists of (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin-gallate (Sarneckis *et al.*, 2006; Schofield, 2001). These building blocks start to polymerize with each other and are then called proanthocyanidins or hydrolysable tannins (Sarneckis *et al.*, 2006; Schofield, 2001).

The colour of grapes comes from the anthocyanins. In its most basic form these anthocyanins are called anthocyanidins, but when it binds with glucose anthocyanins are formed. There are five types of anthocyanins namely cyanidin, delphinidin, peonidin, petunidin and malvidin. Each of these anthocyanins can also be acylated with coumaric acid and acetate (Monagas *et al.*, 2005).

There are different external factors that will influence the concentration of anthocyanins and tannins in the grape berry. The two external factors that go hand in hand are temperature (Jackson and Lombard, 1993, Mori *et al.*, 2005; Chorti *et al.*, 2010) and

sunlight (Kennedy *et al.*, 2000a; Pastor del Rio and Kennedy, 2006). If the ambient day temperature is below 17°C and the night temperature is below 15°C (Jackson and Lombard, 1993) no anthocyanins will be produced to accumulate resulting in less colour in the grape berry (Mori *et al.*, 2005; Chorti *et al.*, 2010). Sunlight is also very important as it helps the flavonoid pathway to produce more anthocyanins (Dokoozlian and Kliewer, 1996). Therefore the row direction and canopy management are very important factors to consider when red grape cultivars are planted. Water is also an important external factor to consider as too much water will dilute the anthocyanin resulting in wine with poor colour (Hardie and Considine, 1976 & Matthews and Anderson, 1988).

Anthocyanins accumulate in the vacuoles of the epidermic cells of the grape berry skin (Ortega-Regules *et al.*, 2006). Anthocyanins are more easily released from the vacuoles than the proanthocyanidins (Ortega-Regules *et al.*, 2006). Proanthocyanidins bind with cell wall components and need enzymes to be released (Arnous and Meyer, 2009). There are different practices and methods to obtain wine with more colour and tannin structure. Methods like cold soaking/maceration (McMahon *et al.*, 1999, Gomez-Plaza *et al.*, 2000, 2001, Alvarez *et al.*, 2009; Gil-Munoz *et al.*, 2009) could release more colour from grape skins, while post maceration (Zimmer *et al.* 2000; 2002) could release more tannins as the alcohol will help in releasing the tannins. Thermovinification (Lowe *et al.*, 1976, Sacchi *et al.*, 2005; Baiano *et al.*, 2009) and carbonic maceration (Gomez-Miguez *et al.*, 2004, Sacchi *et al.*, 2005; Etaio *et al.*, 2008) can also be used to extract anthocyanins and tannins from the skins. These methods will have variable success as wines made from carbonic maceration will have lighter colour but will be fruitier (Etaio *et al.*, 2008).

The proanthocyanidin concentration in wine influences the mouth feel of the wine, especially in terms of astringency and bitterness (Monagas *et al.*, 2005, Gawel, 1997). Furthermore, proanthocyanidins help to stabilize colour (Monagas *et al.*, 2005) when it binds to anthocyanins and increase maturation potential (Lorenzo *et al.*, 2005).

In order to manage anthocyanin and tannin levels in wine, one must be able to measure it. There are three main methods to analyze tannins. These are colorimetric (Schofield *et al.*, 2001), gravimetric (Ginger-Chavez *et al.*, 1997) and precipitation methods (Hagerman and Butler, 1978, Harbertson, 2003; Sarneckis 2006). The first and second methods are not commonly used in the wine industry, but the precipitation methods are

widely used. These include the MCP (methyl cellulose) (Sarneckis *et al.*, 2006) and BSA (bovine serum albumin) methods (Harbertson, 2003). There are also several methods for the analysis of anthocyanins, namely the Iland method (Iland, 2000) for total anthocyanin, Somers & Evans (Rivaz-Gonzalo *et al.*, 1992), Boulton & Levensgood (Levensgood and Boulton, 2004) for copigmentation analysis and Ribereau-Gayon & Stonestreet (Rivaz-Gonzalo *et al.*, 1992) for determining the concentration of anthocyanins.

This literature review will follow the metabolic precursors from photosynthesis to flavonoid biosynthesis. Subsequently the extraction of anthocyanins and proanthocyanidins from the berry to the wine will be discussed. The review will conclude with a discussion of the different analytical methods that can be used for the determination of proanthocyanidins and anthocyanins.

## **2.2 ORIGINS OF TANNIN PRECURSORS**

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To be able to understand proanthocyanidins and anthocyanins in wine, it is important to investigate the origin of these compounds. Knowledge as to how and where the precursors for proanthocyanidins are synthesized, are very important for the understanding of the ultimate role of proanthocyanidins, and also the role they play in the ripening berry. Understanding the external factors that influence these proanthocyanidins will also help the viticulturists in managing proanthocyanidins and anthocyanins concentration in the grape berry.

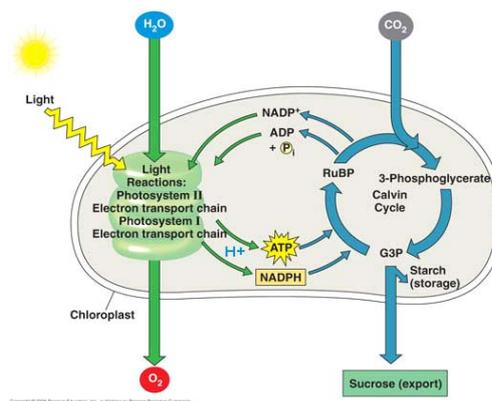
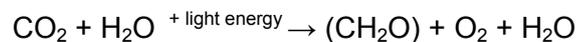
### **2.2.1 Photosynthesis**

It is normally accepted that the state of dormancy are terminated when the mean daily temperatures drops below 10°C for at least 7 consecutive days (Lavee & May, 1997). Budburst takes about 30 days for Shiraz, 35 days for Mataro (Mourvedre) and 32 days for Grenache after the termination of dormancy to occur (Lavee & May, 1997).

During the growing season the vine produce carbohydrates in the form of sugars (more specifically glucose), some of which the vine stores as starch in its shoots (Burger & Deist, 1981 & Winkler, 1965). In the winter this starch is converted to sugar, which in turn, prevents the cells from freezing (Burger & Deist, 1981 & Winkler, 1965). Before the

next growing season, the vine converts the sugar back to starch (Burger & Deist, 1981 & Winkler, 1965). It is this stored carbohydrate which the vine will use for energy and budbreak. This stored energy is used until the vine can start to photosynthesize.

Photosynthesis is a metabolic pathway that converts light energy into chemical energy (Voet & Voet, 2004) and which takes place in the plastids known as chloroplasts (Fig. 2.1) that is found in the leaves of plants. During photosynthesis carbon dioxide (CO<sub>2</sub>), which is taken from the air, and water (H<sub>2</sub>O) which is taken up by the root system, are fixed by sunlight energy (electromagnetic radiation) to yield carbohydrates (a triose phosphate compound called 3-phosphoglycerate, abbreviated as G3P or 3PG, and oxygen (O<sub>2</sub>) (Chen and Zhang, 2008).



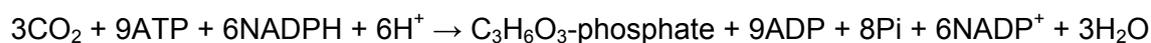
**Figure 2.1** Diagram of photosynthesis in the chloroplast of a leaf (<http://mrskingsbioweb.com/images/10-20-PhotosynthesisRev-L.gif>).

Photosynthesis occurs in two steps:

A **light-dependant reaction step (light reaction)**, where H<sub>2</sub>O is oxidized and where ATP and NADPH are formed (Voet & Voet, 2004). This occurs when the chlorophyll absorbs sunlight and split the water molecule (H<sub>2</sub>O) into hydrogen (H<sub>2</sub>) and oxygen (O). The oxygen molecule is not needed and is released back into the air. The hydrogen dissolves, as a free ion, into the cytoplasm. The energized e<sup>-</sup>, which was removed from the H<sub>2</sub>O molecule, is passed along an electron transport chain to NADP<sup>+</sup> generating NADPH. In the process ADP is phosphorelized to ATP. Both the NADPH and ATP are used in the Calvin cycle (Jackson, 1994).



A **light-independent reaction step (dark reaction)**, where the high energy molecules, ATP and NADPH, are used to fixate CO<sub>2</sub> to synthesize the precursors for carbohydrates through the Calvin cycle or the reductive pentose phosphate cycle (Voet & Voet, 2004).



Therefore it is important to note that photosynthesis is affected by the CO<sub>2</sub> concentrations (Jackson, 1994), temperature, water stress (Hardie & Consideine, 1976), diseases, humidity and light intensity (Jackson, 1994) as well as the nutrients available in the soil.

## 2.2.2 The Calvin cycle (Pentose phosphate cycle)

The Calvin cycle takes place in the chlorophyll plastid (Kruger & von Schaewen, 2003) and consists of two distinct phases.

In the first phase, the oxidative phase, two molecules of NADP<sup>+</sup> are reduced to two molecules of NADPH. The energy for this reaction comes from the conversion of glucose-6-phosphate into ribulose-5-phosphate.



In the second phase, the reductive phase, ribulose-5-phosphate is enzymatically reduced (as shown in Table 2.1) into different metabolites that are used in nucleotide synthesis and phenylpropanoid production (Kruger & von Schaewen, 2003 & Voet & Voet, 2004).

**Table 2.1** List of enzymes in the Pentose phosphate cycle that produce the metabolic intermediate for the amino acid phenylalanine, their EC number (Enzyme Commission number) and the mode of working.

Enzyme	EC number	Mode of working
Phospho-ribulose kinase	2.7.1.19	Ribulose-5-phosphate + ATP = Ribulose-1,5- bisphosphate
Ribulose bisphosphate carboxylase	4.1.1.39	Ribulose-1,5-bisphosphate + CO <sub>2</sub> = 3- Phosphoglycerate
Phosphoglycerate kinase	2.7.2.3	3-Phosphoglycerate +ATP = 1,3-Bisphosphoglycerate
Glyceraldehyde-3-phosphate-dehydrogenase	1.2.1.12	1,3-Bisphosphoglycerate+NADPH=G-3-phosphate
Triose-phosphate isomerase	5.3.1.1	Glyceraldehyde-3-phosphate = Dihydroxyacetonephosphate
Aldolase	4.1.2.14	Dihydroxyacetonephosphate = Fructose-1,6- bisphosphate
Fructose bisphosphatase	3.1.3.11	Fructose-1,6-bisphosphate = Fructose-6-phosphate
Transketolase	2.2.1.1	Fructose-6-phosphate = Erythrose-4-phosphate

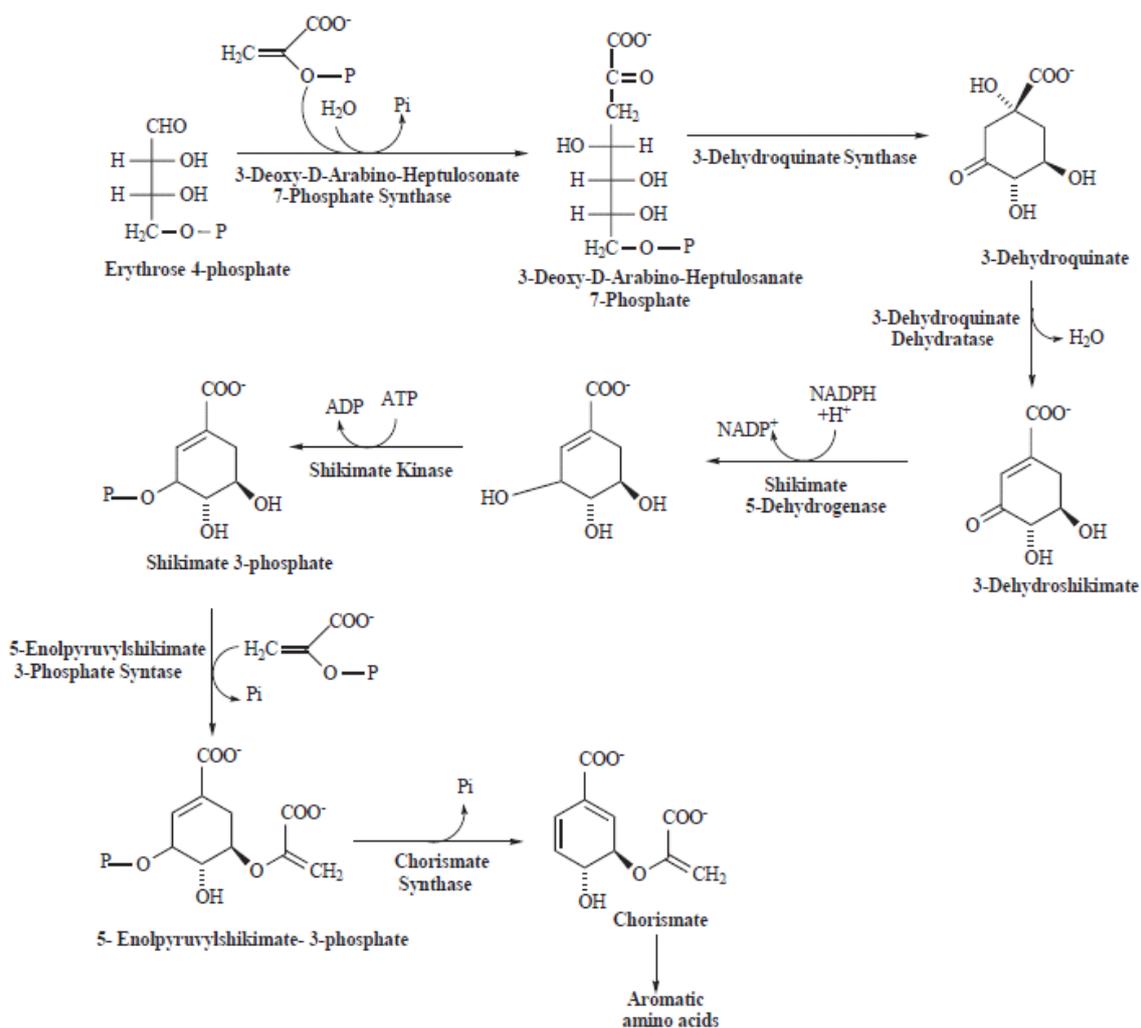
### 2.2.3 Phenylalanine synthesis

To form the aromatic amino acid phenylalanine, erythrose-4-phosphate condenses with phosphoenolpyruvate (PEP), which is obtained from glycolysis, to form chorismate (Fig 2.2). In a further three enzymatic reaction steps, chorismate is turned into the aromatic amino acid phenylalanine. Table 2.2 shows the enzymes that are used to synthesize phenylalanine (Voet & Voet, 2004).

Up until now it has been shown that, as soon as the vine starts to form leaves (E-L 4 stadium) and starts to photosynthesize, the vine begin to synthesize metabolites that it can use during its growing stage. These metabolites will not just give the plant the energy to grow, but also help to protect it against foraging by herbivores (Bogs *et al.*, 2005; Bogs *et al.*, 2007; Jaakola *et al.*, 2002).

The synthesis of phenylalanine only occurs in plants and microorganisms and therefore this pathway is a natural target for herbicides that will not be toxic to man, animal and birds. For instance the active ingredient for Round-Up is glyphosate ( $^{-2}\text{O}_3\text{P-CH}_2\text{-NH-CH}_2\text{-COO}^{-}$ ) which inhibit the forming of 5-enolpyruvylshikimate-3-phosphate in plants (Marques *et al.*, 2007). Necessary amino acids cannot be formed and therefore the plant then dies (Voet & Voet, 2004).

The synthesis of chorismate is also known as the shikimate pathway. Although the original advantage of the phenylpropanoid pathway is still obscure, further studies have shown that the phenylpropanoids serves as key chemical modulators for plant communication with insects and microbes, playing attractive (colour of berries) as well as repellent (phytoalexin responses) roles. The product of the phenylpropanoid pathway is the flavonoids, which gives the plant protection against harmful UV-rays of the sun as well as making the plant unappetizing for herbivores to eat (Ferrer *et al.*; 2008).



**Figure 2.2** Shikimate pathway (Marques *et al.*, 2007).

**Table 2.2** Enzymes that are used to synthesize phenylalanine. The EC number and mode of working are also included.

Enzymes	EC number	Mode of working
2-keto-3-deoxy-D-arabinoheptulosonate-7-phosphate synthase	2.5.1.54	$\text{PEP} + 4\text{EP} = 2\text{-keto-3-deoxyarabinoheptulosonate-7-P}$
Dehydroquininate synthase	4.2.3.4	$\text{DAHP} + \text{NAD}^+ = 5\text{-dehydroquininate}$
5-dehydroquininate dehydratase	4.2.1.10	$5\text{-dehydroquininate} = 5\text{-dehydroshikimate}$
Shikimate dehydrogenase	1.1.1.25	$5\text{-dehydroshikimate} + \text{NADH} = \text{shikimate}$
Shikimate kinase	2.7.1.71	$\text{Shikimate} + \text{ATP} = \text{shikimate-5-phosphate}$
5-enolpyruvylshikimate-3-phosphate synthase	2.5.1.19	$\text{shikimate-5-phosphate} = 5\text{-enolpyruvylshikimate-3-phosphate}$
Chorismate synthase	4.2.3.5	$5\text{-enolpyruvylshikimate-3-phosphate} = \text{chorismate}$
Chorismate mutase	5.4.99.5	$\text{Chorismate} = \text{prephenate}$
Prephenate dehydratase	4.2.1.51	$\text{Prephenate} = \text{phenylpyruvate}$
Aminotransferase	2.6.1.1	$\text{Phenylpyruvate} = \text{phenylalanine}$

## 2.2.4 Phenylpropanoid pathway

The first part of the phenylpropanoid pathway consists of three enzymatic steps. In the first of the enzymatic steps (as shown in Table 2.3), phenylalanine is deaminated by the enzyme phenylalanine ammonia lyase (PAL) to form cinnamic acid. A second enzyme, cinnamic acid 4-hydroxylase (C4H), catalyzes the introduction of a hydroxyl group (-OH) at the *para*-position of the phenyl ring to form *p*-coumaric acid. Then a third enzyme, *p*-coumaric:CoA ligase (4CL), combines a co-enzyme (CoA) to the *p*-coumaric acid to form the *p*-coumaroyl-CoA. Chalcone synthase (CHS) catalyzes the condensation and also the subsequent intermolecular cyclization of three acetate units onto the *p*-coumaroyl-CoA (Ferrer *et al.*, 2008). The full phenylpropanoid pathway is shown in Fig. 2.3.

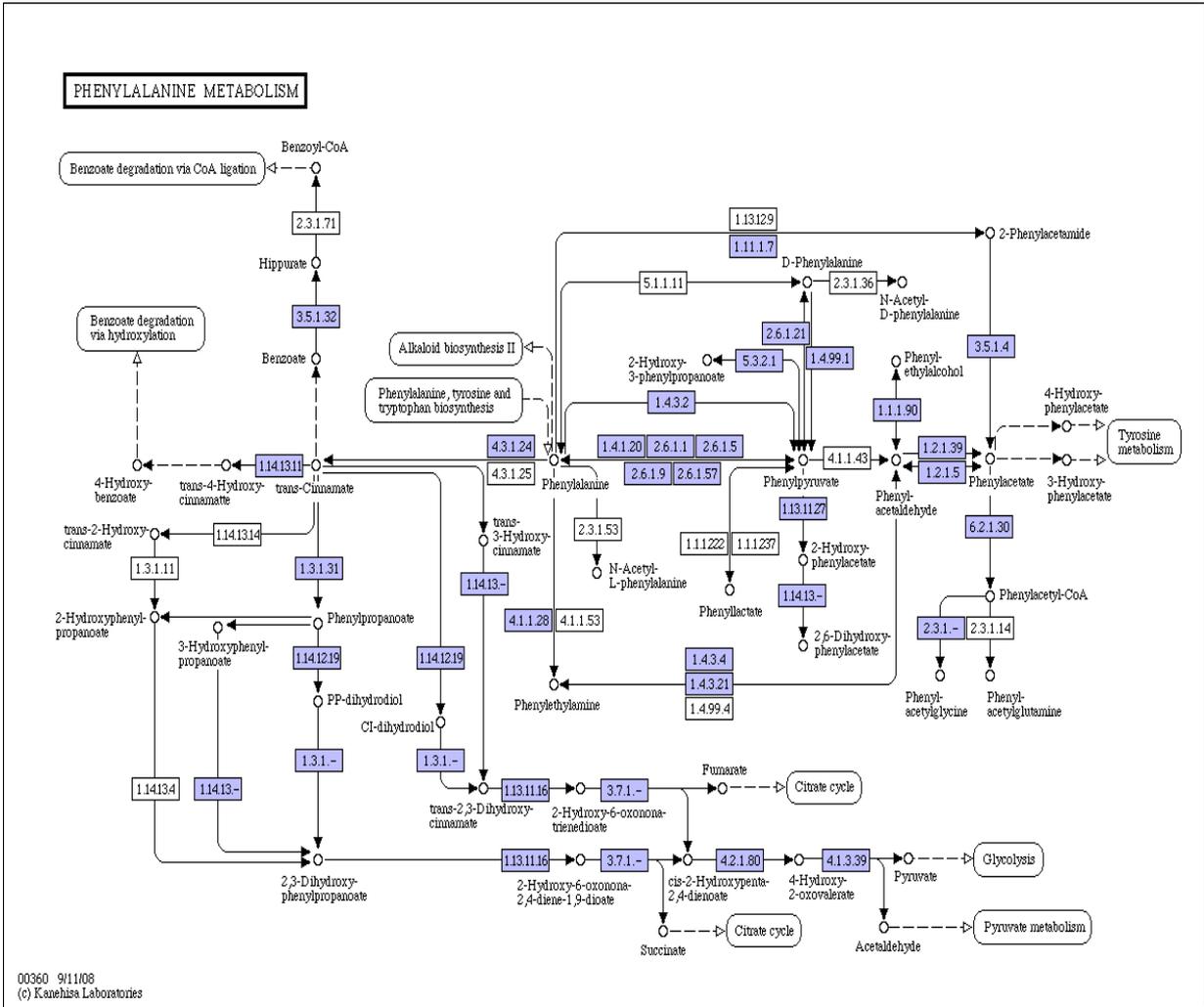
**Table 2.3** The enzymes in the first part of the phenylpropanoid pathway

Enzymes	EC number	Mode of working
Phenylalanine ammonia lyase	4.3.1.5	Phenylalanine = cinnamic acid
Cinnamic acid 4-hydroxylase		Cinnamic acid = <i>p</i> -coumaric acid
<i>p</i> -coumaroyl-CoA ligase		<i>p</i> -coumaric acid + CoA = <i>p</i> -coumaroyl-CoA
Chalcone synthase	2.3.1.74	<i>p</i> -coumaroyl-CoA + malonyl-CoA = chalcone

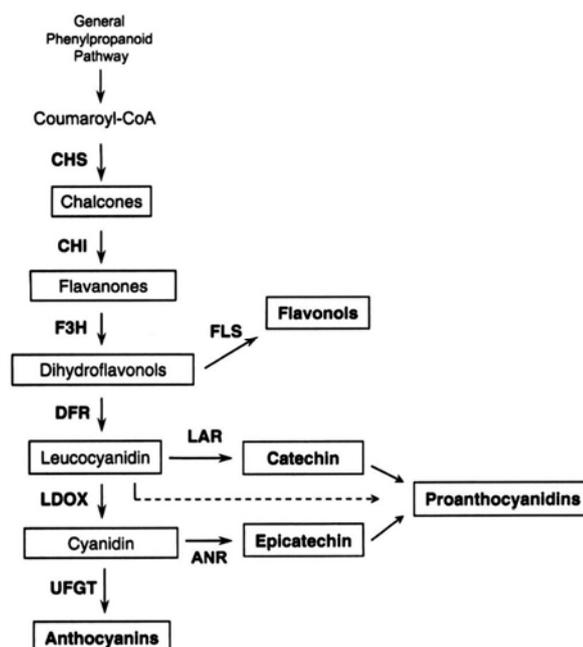
From here the chalcone will be part of the flavonoid biosynthetic pathway where all of the flavonoids are derived from. Only the flavonoids that are relevant to this research will be mentioned in this review.

## 2.2.5 Flavonoid biosynthetic pathway

The final biosynthetic pathway for flavonoids is the flavonoid biosynthetic pathway (Fig. 2.3). During this pathway chalcone is isomerized into naringenin by the chalcone isomerase (CHI) enzyme (Boss *et al.*, 1996 & Winkel-Shirley, 2001). This naringenin is a flavanone. A hydroxyl (-OH) group is then introduced, which binds to the naringenin with help of the enzyme flavanoid-3- $\beta$ -hydroxylase (F3H) to form a dehydrokaempferol (Winkel-Shirley, 2001). By further enzymatic reactions (Table 2.4) the basic building blocks for proanthocyanidins (catechins and epicatechins) and the anthocyanidins are formed.



**Figure 2.3** A schematic overview of the phenylpropanoid pathway in the grapevine



**Figure 2.4:** The flavonoid biosynthetic pathway. The enzymes that are involved in the pathway are as follows: CHS – chalcone synthase, CHI – chalcone isomerase, F3H – flavanone-3- $\beta$ -hydroxylase, FLS – flavonols synthase, DFR – dihydroflavonol-4-reductase, LAR – leucoanthocyanidin reductase, LDOX – leucoanthocyanidin dioxygenase, ANR – anthocyanidin reductase and UFGT – UDP-Glc:flavonoid-3-O-glycosyltransferase (Bogs *et al.*, 2007).

**Table 2.4** The enzymes of the flavonoid biosynthetic pathway

Enzymes	EC number	Genes	Mode of working
Chalcone isomerase	5.5.1.6	VvCHI	Naringenin chalcone = naringenin
Flavanone-3- $\beta$ -hydroxylase			Naringenin = dihydrokaempferol
Flavonoid-3'-hydroxylase			Dihydrokaempferol = dihydroquercetin
Flavonoid-3',5'-hydroxylase		VvF3'5'H1	Dihydrokaempferol = dihydromyricetin
Flavonol synthase			Dihydroquercetin/dihydromyricetin = uercetin/myricetin
Dihydroflavonol-4-reductase	1.1.1.219		Reduce the dihydroflavonols to leucoanthocyanidins
Leucoanthocyanidin reductase		VvLAR1	Reduction of the leucoanthocyanidin to its corresponding anthocyanin
Leucoanthocyanidindioxygenase		VvLDOX	Catalyze the synthesis of anthocyanins
Anthocyanidin synthase			leucocyanidin/delphinidin = cyanidin/delphinidin
Anthocyanidin reductase	1.3.1.77	VvANR	Cyanidin = epicatechin
UDP-Glc:flavonoid-3-O		VvUFGT	cyanidin/delphinidin = different anthocyanins-glucosyltransferase
Methyltransferase	2.1.1.6		Glucosylation of glucose to the anthocyanins

## 2.3 TRANSLOCATION AND DEVELOPMENT OF TANNIN AND ANTHOCYANIN

Flavonoid synthesis takes place inside the berry as the berry is a sink for minerals and monosaccharides (Coombe, 1992). The berry has two important organs where flavonoid metabolites can accumulate, i.e. the skin and the seed of the berry.

There are two classes of genes that are required for biosynthesis, namely the structural genes (the genes encoding the enzymes that directly participate) and the regulatory genes (the genes that control the transcription of the structural genes). Therefore the enzyme activity in the various pathways is highly regulated (Jaakola *et al.*, 2002).

According to a study done by Bogs *et al.* (2007), the grapevine transcription factor VvMYBPA1 helps to regulate two of the structural genes (LAR and ANR) in the flavonoid biosynthetic pathway that catalyze the transformation of proanthocyanidins and anthocyanidins. They found that development of the grape berry occurs in two stages. In the first stage, which is from flowering to veraison, VvMYBPA1 regulates the proanthocyanidins (PA) synthesis. In the second stage, the onset of ripening, VvMYBA1 regulates anthocyanidin synthesis (Bogs *et al.*, 2005, 2007).

### **2.3.1 SKIN TANNIN**

The expression of VvLDOX decreases from six weeks before veraison to low levels just before veraison and then increases significantly following veraison (Bogs *et al.*, 2005). Expression of VvANR also decreases from six weeks prior veraison and was not detected after veraison (Bogs *et al.*, 2005). VvLAR1 was detected four weeks before veraison but not later on in the developing stages, but VvLAR2 increases to a maximum four weeks before veraison, then decreases to low levels at veraison and maintained the levels throughout berry ripening (Bogs *et al.*, 2005).

The proanthocyanidins (PA) forms polymers of between 25-40 subunits, which consists of equal proportions of (-)-epicatechin and (-)-epigallocatechins with (+)-catechins as terminal subunits (Bogs *et al.*, 2005; Downey *et al.*, 2003 & Kennedy *et al.*, 2000b). The polymer length remained constant at about 30 to 40 subunits until veraison at which point it decreased slightly too about 30 subunits four weeks after veraison. The polymer length then drop until approximately 20 subunits at harvest (Downey *et al.*, 2003). Figures 2.8 and 2.9 shows probable diagrams of proanthocyanidins with extension and terminal subunits.

The transcription levels of VvMYBPA1 are low in the grape berry skins before veraison and increases to a maximum about two weeks after veraison after which they decline to low levels again. (Bogs *et al.*, 2007). Therefore the proanthocyanidins in the skins

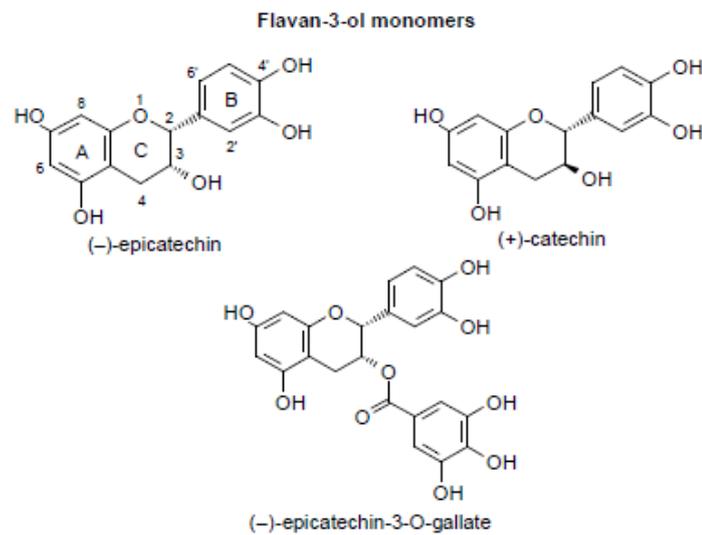
increases from five weeks before veraison to a maximum concentration about two weeks after veraison and then decline during ripening (Bogs *et al.*, 2007).

### 2.3.2 SEED TANNIN

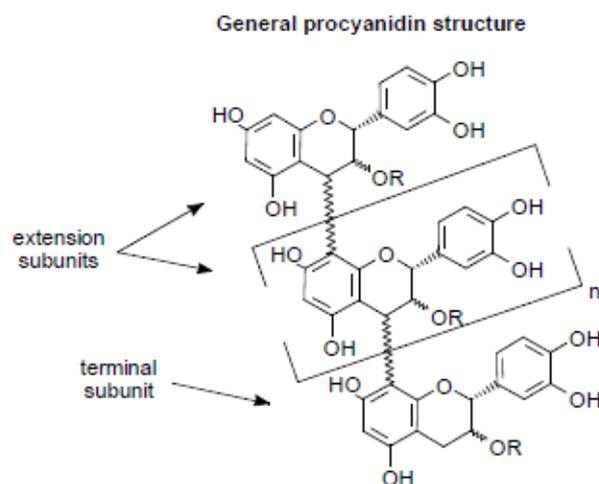
In the seeds the transcription factor VvMYBPA1 are expressed before veraison when the proanthocyanidins starts to accumulate (Bogs *et al.*, 2007). The expression of VvLDOX and VvANR reach a maximum six weeks before veraison where it plato at a constant level until veraison after which it decreases to low levels during ripening (Bogs *et al.*, 2005 & 2007). VvLAR1 expresses six weeks before veraison then decreases to low levels, whereas the expression of VvLAR2 increases to a maximum at veraison and then decreases during ripening (Bogs *et al.*, 2005). Proanthocyanidin synthesis occurs in the developing flower before pollination and it also shown that most of the flavonoid genes, of the flavonoid biosynthetic pathway, are expressed at flowering (Bogs *et al.*, 2005) and that the proanthocyanidins increases to a maximum just after veraison where the levels stayed relatively constant and then decreases during ripening (Bogs *et al.*, 2005).

The mean degree of polymerization (mDP) of the seeds was 4 to 6 subunits and comprises of the following: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and (-)-epicatechin-gallate (Bogs *et al.*, 2005 & Downey *et al.*, 2003). In a study done by Downey *et al.* (2003) on Shiraz in Southern Australia, they found that (+)-catechin, (-)-epicatechin and (-)-epicatechin-gallate, as shown in figures 2.5 and 2.6, are all found as terminal subunits (Kennedy *et al.*, 2000b) and was confirmed by Downey *et al.* which also did the study on Shiraz (Downey *et al.*, 2003). (-)-Epicatechin is the major constituent at 65% of the extension subunits, while (-)-epicatechin-gallate and (+)-catechin make out 25% and less than 10% respectively (Downey *et al.*, 2003). They also found that in the 2000-2001 seasons the seed weight increased for approximately four weeks and then slowed down. It reached a maximum weight one to two weeks before veraison and then declined to about 20-30% until harvest (Downey *et al.*, 2003). The extension subunits showed a rapid increase from fruit set until four weeks before veraison where after the levels stayed relatively constant until two weeks after veraison (Downey *et al.*, 2003). The levels stayed relatively constant until eight weeks after veraison with a final decrease to harvest (Downey *et al.*, 2003). The terminal subunits also shown a rapid increase from fruit set until one to two weeks before veraison when it

slowed down. A quick increase over veraison took place to reach a maximum at one to two weeks after veraison after which it declines until harvest (Downey *et al.*, 2003).



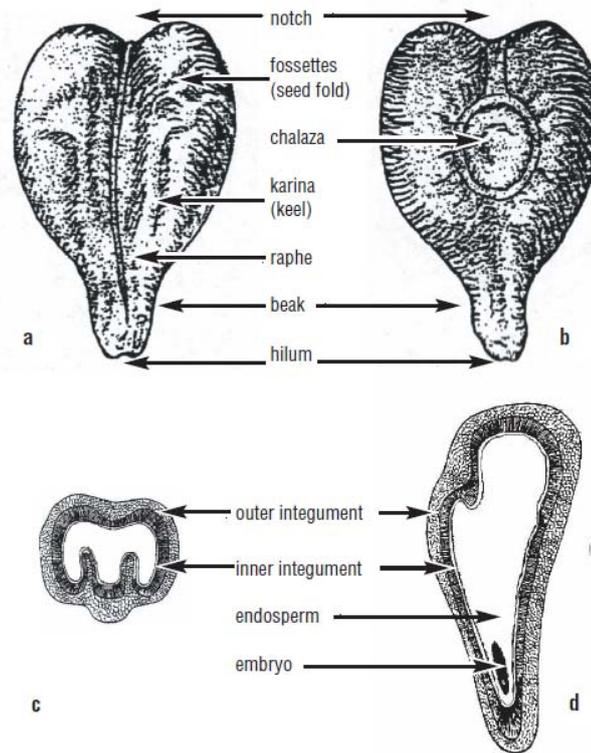
**Figure 2.5:** The three flavan-3-ol monomers that are found in grape seeds (Kennedy *et al.*, 2000b)



**Figure 2.6:** Probable diagram of procyanidins which contain extension and terminal units of (+)-catechin (C), (-)-epicatechins (EC) and (-)-epicatechin gallate (ECG) (Kennedy *et al.*, 2000b).

### 2.3.3 GRAPE SEED

The grape seed consists of three principle tissues: (i) the seed coat or testa (consisting of the outer and inner integument), (ii) an endosperm (containing oil, a protein called aleurone and calcium oxalate crystals) and (iii) an embryo (Figure 2.7, Ristic and Iland, 2005).



**Figure 2.7:** Line diagram of the ventral (a) and dorsal (b) sides of a mature grape seed showing the beak, hilum, notch, fossettes, karina, raphe and chalaza. Central transversal (c) and longitudinal (d) section of a grape seed showing the outer and inner integument, endosperm and embryo (adapted from Ristic and Iland, 2005)

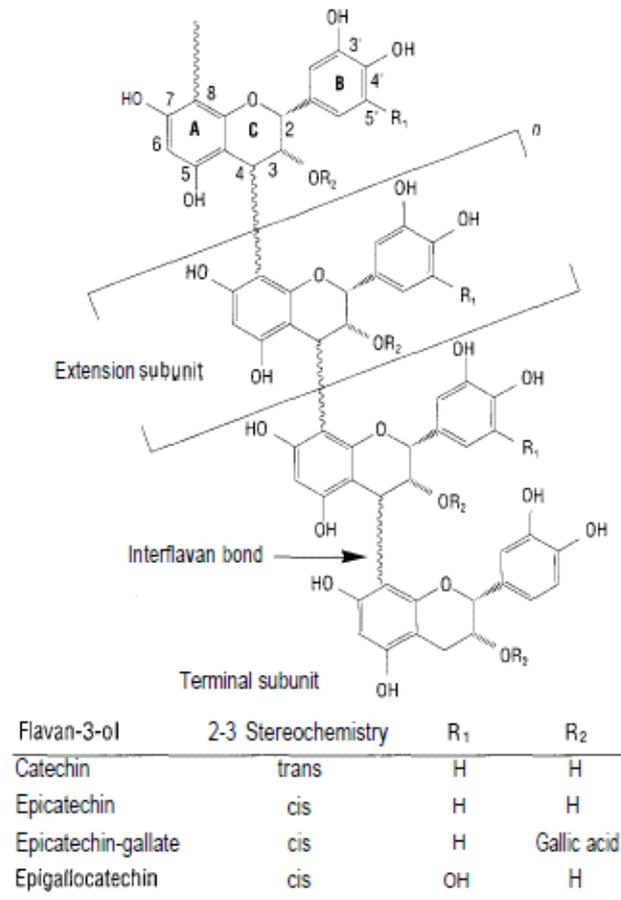
There are three phases of seed growth and development: (1) a seed growth phase that is characterized by an increase in both the fresh and dry weight, the synthesis and accumulation of flavan-3-ols and tannins and a green appearance of the seed, (2) a transitional phase where the fresh and dry weight of the seeds reached a maximum, but with a continuing enlargement of the basal end, the accumulation of flavan-3-ols and tannins reached a maximum, an oxidation of the tannins take place accompanied with a yellow appearance of the seed and (3) a seed drying and maturation phase where the fresh weight decreases due to water loss, a further oxidation of tannins and an overall brown appearance (Ristic and Iland, 2005).

There are three developmental stages in the maturation of a grape berry. The first stage is characterized by an herbaceous growth phase that lasted for 45 to 65 days. The growth hormones (cytokinins and gibberellins) correspond directly with the number of seeds. The intensity of cellular multiplication depends on the existence of the seeds. Cellular growth begins about two weeks after fertilization and continues until the end of the first phase. Chlorophyll is the predominant colour and the berries have intense

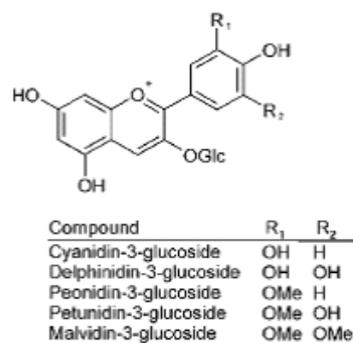
metabolic activity that is characterized by an elevated respiratory intensity and a rapid accumulation of acids (Ribereau-Gayon *et al.*, 2000). The second stage is characterized by the colouring of red grapes called veraison. This phase can be 8 to 15 days long. An increase in abscissic acid takes place (Ribereau-Gayon *et al.*, 2000). It is in this first phase of seed growth and the second stage of berry development, that the bulk of procyanidins are synthesized (Kennedy *et al.*, 2000 & Ristic and Iland, 2005). It is during this phase of flavan-3-ol synthesis and procyanidins accumulation (Figure 2.8) that the green berry has the highest concentration of seed tannin. It reaches a maximum around veraison, which is also the onset of stage three of berry development (Kennedy *et al.*, 2000b & Ristic and Iland, 2005). The third growth stage corresponds to maturation of the grape berry. The respiratory intensity decreases, while the enzymatic activities increases. This stage can last for 35 to 55 days during which the grape berry accumulates sugars, cations ( $K^+$ ), amino acids and phenolic compounds, while the concentration of malic acid and ammonium decreases (Ribereau-Gayon *et al.*, 2000). Therefore seed maturity can be defined as a state of dehydration where the accumulation of food are complete and the dry weight has reached its maximum (Ristic and Iland, 2005). Ristic and Iland (2005) also mentioned that there was a good correlation between seed colour value and corresponding changes in phenolic composition and that the colour of the seed can relate to berry ripeness.

#### **2.3.4 ANTHOCYANIN IN THE GRAPE SKIN**

There are five basic anthocyanidins, which are shown in figure 2.9. These anthocyanins accumulate in the vacuoles in the upper cellular layers of the hypodermis of the berry skin (Gonzales-Neves *et al.*, 2008).



**Figure 2.8:** A probable diagram of proanthocyanidin with the flavan-3-ols (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC) and (-)-epicatechin-gallate (ECG) of which the skin tannins are comprised of (Downey *et al.*, 2003).



**Figure 2.9:** Different types of anthocyanins (Jensen *et al.*, 2008).

The total amount of anthocyanins at harvest depends on a couple of agro-economical factors including variety, environmental factors (i.e. climate) and agronomical practices (i.e. pruning, irrigation, canopy management etc.) (Rolle *et al.*, 2009 & Rio Segade *et al.*, 2008). The tannins and anthocyanins form different complexes with the cell wall components during berry development (Geny *et al.*, 2003). As the berry ripens these complexes are broken up more easily than unripe berries.

The transcription factor VvMYBA are normally switched off before veraison and only starts to express after veraison (Bogs *et al.*, 2005 & 2007). VvMYBA encodes for the LDOX enzyme, which catalyze the synthesis for anthocyanin (Bogs *et al.*, 2005 & 2007) with UFGT, which is encoded by VvUFGT (Bogs *et al.*, 2005). Anthocyanin synthesis occurs after proanthocyanidin accumulation is completed (Bogs *et al.*, 2005 & 2007 & Downey *et al.*, 2003).

It is therefore evident that the anthocyanins are synthesized in the berry at the beginning of veraison. The anthocyanidins then binds with a glucose molecule, which is transported to the berry via phloem sap flow (Coombe, 1992), to form anthocyanin. The anthocyanins are then translocated to the vacuoles of the epidermic cells of the grape berry skin.

Thus the highest concentration of proanthocyanidins (Figure 2.8) occurs just before veraison with a decrease until harvest. Although the total tannin concentration can be higher in seeds than in skins, the polymer length is found to be higher in the skins (Downey *et al.*, 2003). Therefore the seed procyanidins will be more astringent than skin proanthocyanidins to deter animals eating the berries before ripening.

### **2.3.5 ENVIRONMENTAL FACTORS INFLUENCING THE SYNTHESIS OF GRAPE TANNIN AND ANTHOCYANIN**

#### **2.3.5.1 SUNLIGHT**

Different environmental factors like sunlight, temperature and plant water status play a role in the accumulation of proanthocyanidins in a developing grape berry (Kennedy *et al.*, 2000a & Pastor del Rio and Kennedy, 2006). If the bunch is exposed to sunlight during growth stages I and II, the enzyme phenylalanine ammonia lyase, increases and therefore the concentration of phenols and anthocyanins will be higher. Light is needed to maintain the production of these enzymes throughout berry development (Dokoozlian and Kliewer, 1996).

Different studies showed varied results pertaining to the effect of sunlight on anthocyanin and tannin concentration during ripening. Haselgrove *et al.* (2000) investigated the effect of high sunlight exposure of Shiraz berries on their phenolic composition. They

found that berries that received high levels of sunlight had high levels of quercetin-3-glucoside and low levels of malvidin-3-glucoside. Therefore higher light intensities promote greater accumulation of anthocyanins but the anthocyanin accumulation depends also on the range of light intensity. In another study done by Spayd *et al.* (2002) on Cabernet Sauvignon grapes, the effect of sunlight on the total skin monomeric anthocyanins (TSMA) was tested. They found that the Cabernet Sauvignon grapes that were exposed to sunlight increased their TSMA concentration regardless of the ambient temperature. The cooling (sun-blower) of sun-exposed grapes increased the TSMA, while the heating (shade-blower) decreased the TSMA in 1999 but had no effect in 2000. They also found that UV-light barriers reduced individual and total flavanol concentration.

On the other hand, Crippen and Morrison (1986) found that there was no significant difference in anthocyanin concentration at harvest time from sun-exposed to shaded grapes, although the concentration of anthocyanin was higher throughout berry development in the sun-exposed grapes. The percentage of polymerized phenols was higher in the shaded grapes. Ristic *et al.* (2007) found that the amount of anthocyanins of Shiraz bunches, that was enclosed in a special designed box, was not significantly different from that of the unshaded bunches. The only difference was that the anthocyanin composition of the shaded bunches shifted towards dioxygenated anthocyanins. The shaded bunches also had increased seed tannins and decreased skin tannins.

#### **2.3.5.2 TEMPERATURE**

Jackson and Lombard (1993) found that the optimum day temperature for berry colouration is between 17°C to 26°C and the optimum night temperature is between 15°C to 20°C. This has been confirmed by Mori *et al.* (2005) and Chorti *et al.* (2010) where they found that high night temperatures decrease the anthocyanin accumulation in the berry (Mori *et al.* 2005) and that many metabolic processes stop or slow down when the ambient temperature get to 30°C (Chorti *et al.* 2010).

Mori *et al.* (2005) also found that although the anthocyanin concentration decreases with high night temperature, there was no effect on the flavanol concentration. The high night temperature inhibited the expression of chalcone synthase (CHS), flavanone-3-

hydroxylase (F3H), dihydroflavonol-4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX) and UDP glucose: flavonoid -3-O-glucosyltransferase (UFGT) which are the key enzymes in the flavonoid biosynthetic pathway.

Harbertson *et al.* (2002) found that the total amount of seed tannin in berries is correlated with the amount of seeds per berry. This was confirmed by Pastor del Rio *et al.* (2006) where they also found that cool temperature during fruit set influences the number of seeds per berry and that cool temperatures during this time increases the amount of tannins with an increase in proanthocyanidins.

Tarara *et al.* (2008) found that as the berry temperature increases the total skin anthocyanin (TSA) decreases. The glucosides of peonidin, petunidin, delphinidin and cyanidin with their acylated (acetic- and coumaric acids) forms decreases but there was no effect on the malvidin-3-O-glucoside and its acylated forms. Although, they distinguish between a dense canopy with high berry temperatures that led to lower malvidin-3-O-glucoside and where their bunch had direct solar radiation with elevated berry temperature with no effect on the concentration of malvidin-3-O-glucoside (Tarara *et al.*, 2008). Chorti *et al.* (2010) found the same i.e. that temperature above 30°C inhibited anthocyanin accumulation. The high berry temperature has more influence on anthocyanin accumulation than sunlight exposure, although the shading of the fruit zone reduces the total soluble solids and anthocyanin accumulation (Chorti *et al.* 2010). Buttrose *et al.* (1971) found that daylight temperature of 20°C promotes colour formation and that at 30°C daylight temperature the colour will be less.

### **2.3.5.3 WATER STRESS**

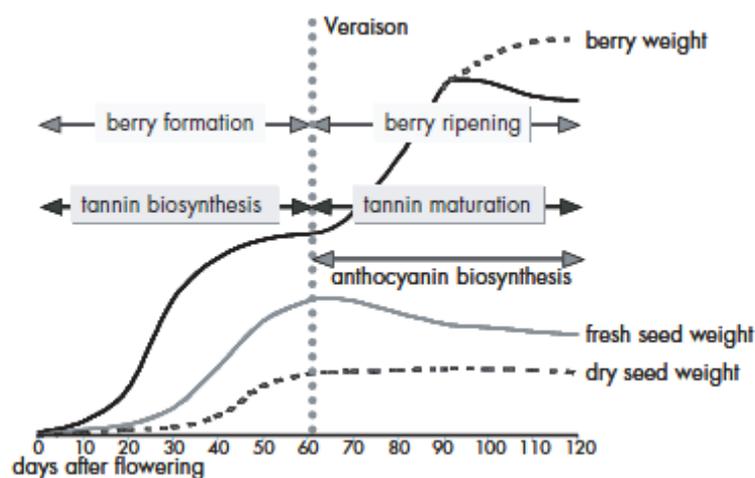
Hardie and Considine (1976) and Matthews and Anderson (1988) found that the colour of the must and wine increases with unirrigated vines. They also found that the accumulation of anthocyanins during veraison is directly correlated with carbohydrate metabolism. That is also the reason why defoliated vines or vines with low leaf area caused poor accumulation of anthocyanins (Hardie and Considine, 1976).

Water stress during veraison can also decrease anthocyanin accumulation as a consequence of reduced carbohydrate availability. The seed number is determined by the number of the pollen tubes that successfully reach and fertilize the ovulus.

Therefore water stress at that stage of berry development may have an effect on the number of seeds per berry and subsequently on tannin concentration (Roby and Matthews, 2004).

## 2.4 EXTRACTION OF TANNINS AND ANTHOCYANINS INTO WINE

The accumulation of anthocyanins and tannins in the developing grape berry is very complex. The study on the phenolic ripeness and extraction of these anthocyanins and tannins is a recent field of study (Glories, 1984, Kennedy *et al.*, 2000 & 2001, Habertson *et al.*, 2002, Herderich *et al.*, 2001 & 2004, Romero-Cascales *et al.*, 2005, Ortego-Regules *et al.*, 2006, Rio Segade *et al.*, 2008; Rolle *et al.*, 2009). Figure 2.10 shows the grape berry development and accumulation of anthocyanins and tannins from flowering up to harvesting.



**Figure 2.10:** Berry formation and ripening and the biosynthesis of anthocyanin and tannin (adapted from Herderich *et al.* 2004).

### 2.4.1 PHENOLIC RIPENESS AND EXTRACTABILITY OF ANTHOCYANINS INTO WINE

From the time that the first grapevine was planted, in approximately 8000 BC in Anatolia (McGovern, 2003), methods to achieve ripeness as soon as possible were employed. There are different ways to test whether the grapes are ripe enough, however the easiest way is to taste the grapes to find out if it is sweet enough to pick and eat. Modern-day winemakers use analytical methods to predict ripeness. According to Bisson (2001) grape maturity can be defined as the physiological age of the berry on the vine. Thus, phenological ripeness will differ from cultivar to cultivar.

In 1984 Yves Glories, from France, established an assay to measure the extractability of the anthocyanin from the grape berry skin. Two hundred berries from a homogenous berry sample are counted, weighed and homogenized. 2 x 54 grams of the homogenized grapes are weight off and mixed with 50 mL of a pH 1 solution and 50 mL of pH 3.2 solutions. These mixtures are shaken and left for 4 hours after which they are filtered. 1 mL of each solution is then mixed with 1 mL of a 96% ethanol solution (with 0.1% HCl) and 20 mL of 2% HCl. From the pH 1 solution 10 mL are taken out and put into a test tube (tt1) with 4 mL of distilled water. From the same solution 10 mL are put into a second test tube (tt2) with 2 mL of distilled water and 2 mL of a metabisulfite solution. The same is done with the pH 3.2 solution (tt3 and tt4). The four test tubes are left for 20 minutes after which the absorbance value is measured at 520 nm.

The following formula is used by Glories (1984) to determine the percentage extractability:

$$d1 = (tt1-tt2)*875*2 \quad 1$$

$$d2 = (tt3-tt4)*875*2 \quad 2$$

$$Total\ Anthocyanin\ (mg/L) = (tt1-tt2)*875*100 \quad 3$$

$$EA\% = [(d1-d2)/d1]*100 \quad 4$$

$$Skin\ tannin = A_{280}^{pH3.2} * 40 \quad 5$$

In this method the absorbance of anthocyanin of homogenized grapes are measured at 520 nm at wine pH (which is for this method pH3.2) and at pH1. At this low pH the cell walls are ruptured and the anthocyanin is extracted. The difference between pH1 and pH3.2 gives the extractability. The lower the values between d1 and d2, the smaller the difference between these two measurements and the more easily extractable the anthocyanins are. The values are normally between 20 – 70% where values smaller than 40% are considered as an indication of phenolic ripeness. The values will differ between different cultivars. Therefore the lower the value the more easily the anthocyanins will be extracted.

The thickness of the skins differs between cultivars, so the values for extractability will differ from cultivar to cultivar. Studies done by Rio Segade *et al.* (2008) and Rolle *et al.* (2009) have shown that the thickness of the berry skin has a large influence on the

extraction of anthocyanins. Table 1.5 show eight cultivars that were tested by Rio Segade *et al.* (2008) and cultivars with thicker skins had more anthocyanin extracted than cultivars with thinner skins. Letaief *et al.* (2008) tested the skin break force (Fsk) of three cultivars namely Cabernet Sauvignon, Pinot noir and Nebbiolo. He found that Pinot noir required more energy to break the skin (0.551 N), than Cabernet Sauvignon (0.421 N), with Nebbiolo (0.398) having the softest skin (Letaief *et al.*, 2008). Mourvedre has very thick skin and have a lower extractability than Merlot which has a thinner skin with a higher extractability. This is a new field of research and studies can be conducted to look at the thickness of the skins of different cultivars from different regions and the subsequent extraction into wine.

**Table 2.5:** Skin thickness, total anthocyanin and extractable anthocyanin of eight Spanish cultivars (adapted from Rio Segade *et al.*, 2008).

Cultivar	2005			2006		
	SPsk <sup>1</sup>	Total Anth (mg/kg) <sup>2</sup>	EA (mg/kg) <sup>3</sup>	SPsk	Total Anth (mg/kg)	EA (mg/kg)
Albarello	213	425	380	270	432	301
Brancellao	170	600	476	233	457	289
Ferron	216	1446	995	213	1589	961
Gran Negro	206	1062	730	188	1005	635
Loureira Tinta	193	2524	1453	230	2660	1344
Merenzao	170	335	312	181	191	116
Mouraton	192	1033	697	240	821	427
Souson	209	1732	1171	164	1913	968

- Berry skin thickness (µm)
- Total anthocyanin (mg/kg)
- Extractable Anthocyanin (mg/kg)

The extractability of anthocyanins increases throughout berry ripening as a consequence of pectolytical degradation of the berry cell wall (Rolle *et al.*, 2009). Therefore, phenolic maturity is reached when the grape anthocyanin concentration is at its maximum (Ortega-Regules *et al.*, 2006). Studies done by Ortega-Regules *et al.* (2006), Rio Segade *et al.* (2008) and Rolle *et al.* (2009) have shown that the extraction of anthocyanin from the cells is a diffusion process and that the rate of the extraction is influenced by the concentration of grape anthocyanin, the composition and thickness of the berry cell walls and the processing methods.

Seed maturity also plays a part of the phenolic maturity of the grapes (Romero-Cascales *et al.*, 2005). It can be calculated by the following formula:

$$MP\% = [(TPI - T_{skin}) / TPI] * 100$$

6

Total phenols are made up of the tannin of the skins as well as the tannins of the seeds and the values are normally between 0-60% (Glories, 1984), the MP decreases with berry ripening, therefore the higher the MP% the more astringent the resulting wine will be. Thus, the MP% shows the contribution of seed tannins to the wine (Romero-Cascales *et al.*, 2005).

#### **2.4.2 BERRY SENSORY ASSESSMENT (BSA)**

In 2004 Erika Winter *et al.* compiled an assessment guide to be used in the vineyards. Many viticulturists and grape growers had their own method of assessing the potential quality or maturity of their grapes in the vineyards. These methods differ from judging the colour of the grapes to chewing and spitting of the grapes to assess pip maturity and colour release.

In order to judge the potential quality or grape maturity of a vineyard, the grape ripening must be even. Therefore, everything starts with good agricultural practices, which include site, variety, rootstock, clone etc. After that the most important prerequisite for grape quality is that the vines must be in balance. Archer and Hunter (2004) defined vine balance in five subgroups, i) Balance between subterranean growth and growth above the surface, ii) balance between fine and thick roots, iii) balance between left and right cordons, iv) balance between shoot growth and yield, and v) balance between young and old leaves in the canopy.

According to the wine grape berry sensory assessment (2004) of Winter *et al.* there are a whole plethora of factors to consider. 1) Examining softness of the berry. An unripe berry will be hard to squash and as the berry ripens the berry became soft and squashable. 2) Assessing the ease with which the stalk can be removed from the berry. Normally the skin around the insertion point will be torn out in unripe berries. In ripe berries, no pulp will remain on the stalk and the “brush” will be clearly visible. 3) Examining the skin colour of red cultivars. Pink skin colour means the berry is at veraison and the darker the skin colour the riper the berry will be. 4) Examining pulp consistency. In unripe berries the pulp will adheres strongly to the skin and seeds. As the berry ripens, there will be no pulp adhered to the skins and seeds and the juice will be released when the berry is squashed. 5) Examining pulp sweetness, acidity and aromas. When the berry is chewed, the sweetness, acidity can be determined. The

grape specific aroma can also be determined post-nasally. 6) Examining skin disintegration. The skins of ripe grapes are easily chewed, but the skins of unripe grapes are difficult to break. 7) Examining skin tannin intensity and astringency. In skins with low levels of phenolic compounds, the tongue will slide effortlessly across the roof of the mouth. So as the grape ripens the tongue will slide over the roof of the mouth with more and more difficulty. Astringency is noticed when there is a reaction between the grape phenolics and saliva in the mouth. The phenols bind to the proteins in the saliva and precipitates and an astringent sensation is noticed. 8) Examining seed colour. Green soft seeds mean that the grapes are unripe. Dark brown seeds are correlated with ripe grapes. 9) Assessing seed moisture and crushability. Green seeds are soft, but seeds of ripe berries are hard and will crack when you bite them.

### **2.4.3 WINEMAKING TECHNIQUES AND THE EXTRACTION OF ANTHOCYANIN AND TANNIN INTO WINE**

From the pre-modern era the grape juice was left to ferment on its own accord. It was only when Louis Pasteur proved in 1865 that a microorganism is responsible for fermentation that modern researchers started to use active dried yeast (Pretorius *et al*, 1998 and McGovern *et al.*, 2005). It was also noted that the juice of red grapes is actually white and not red as one would expect. With these observations came the next important question: what happened during fermentation that caused anthocyanins to be extracted into the wine.

During normal fermentation of red grapes, the grapes are destemmed and crushed without breaking the pips. The juice along with the skins are then inoculated with an active dried yeast culture and left for at least a week to finish fermentation. Afterwards the wine is racked off the skins and the skins are pressed to obtain more of the wine that is captured between the skins. This pressed wine is stored along with the free run wine in a wooden barrel and left for further maturation and malolactic fermentation.

The anthocyanins accumulated in the vacuoles or anthocyanoplasts in the epidermic cells of the grape skin. This anthocyanin can be easily released from the cells when these cell walls are mechanically broken. Tannins form complexes with cell wall components and are therefore more difficult to release as these complexes must firstly be broken.

Herderich *et al.* (2001) found that only about 10%-24% of grape tannins could be detected in the wine and that the concentration of these tannins was related to the winemaking practices. Adams and Scholz (2004) found that about 60% of seed tannin and about 40% of skin tannins are extracted during normal winemaking practices. The distribution of proanthocyanidins of the grape vine is roughly as follows: 66% in the seeds, 45% in the stems, 28% in the leaves and only about 26% are in the skins. If the leaves and stems are crushed and used for the winemaking process, too much tannin will be extracted, which will have a negative effect on the mouth feel of the wine.

#### **2.4.3.1 COLD SOAKING**

Cold soaking/maceration are known as the prefermentation skin contact time. After crushing of the grapes the vacuoles of the skin cells are broken and the anthocyanins can seep out. The absence of alcohol, at that time, allows the formation of higher molecular weight pigmented phenolic species which will enhance colour stability (McMahon *et al.*, 1999).

This can be done at different temperatures and for different lengths of time as a number of studies have shown. Gomez-Plaza *et al.* (2000) used Monastrell grapes to be cooled down to 10°C for 5 days and in 2001 tested three different maceration times (4, 5 and 10 days). In both studies Gomez-Plaza *et al.* found that the low maceration temperature (10°C) and longer maceration time (10 days) had a positive influence on the anthocyanin concentration, chromatic characteristics and hydroxyl cinnamic acid derivatives of the wines. After storage of twelve months the wines made with the longer maceration time (10 days) kept their colour density and phenolic contents for longer than the shorter maceration times (4 days) (Gomez-Plaza *et al.* 2000). Longer skin contact time may lead to greater polymerization of pigments and higher concentrations of procyanidins and therefore to a greater colour stability in the early stages of red wines (Gomez-Plaza *et al.*, 2001). Alvarez *et al.* (2009) used Tempranillo which was cooled to between 5-8°C for 4 days and Gil-Munoz *et al.* (2009) used Cabernet Sauvignon and Shiraz to be cooled down to 10°C for 7 days. They also found that the low maceration temperature gave more concentration of anthocyanins and total phenols.

In a study done by McMahon *et al.* (1999) on Pinot noir it was found that cold soaking at 4°C gave darker less bitter wines, while the Pinot noir at 10°C had less colour and more woody-tobacco aroma with an increase in bitterness. The majority of anthocyanin is extracted after the fermentation of the first 10°B (McMahon *et al.*, 1999), although anthocyanin ionization increases with maceration time (Gomez-Plaza *et al.*, 2001). Tannins continue to be extracted throughout the fermentation process and pressing of the skins prior to dryness enhances fruity character, limits astringency and decreases the total phenol concentration (McMahon *et al.*, 1999). Although a decrease in tannin concentration may limit astringency, a high concentration of tannin may stabilize anthocyanin (more stable colour), but it can also lead to excessive astringency in wines. Reactions involving the stabilization of anthocyanin are the formation of copigmentation (Boulton, 2001), acetaldehyde mediated condensations (Timberlake and Bridle, 1976; Somers and Evans, 1986) and the formation of polymers with tannins.

Parenti *et al.* (2004) tested two different cryogenes, solid state CO<sub>2</sub> and liquid N<sub>2</sub>, on Sangiovese. The cryogenes were used to cool the grapes down to -5°C, 0°C and 5°C for two days. They found that a decrease in cold maceration temperature lead to an increase in extraction of anthocyanins and wine quality, until a point where any further decrease in temperature had no effect on any further extraction of anthocyanins.

#### **2.4.3.2 CARBONIC MACERATION**

Clusters of whole berries are held under a carbon dioxide atmosphere and a partial fermentation occurs because of the activity of glycolytic enzymes that are present in the grapes (Sacchi *et al.* 2005). After a specific period of time, typically one to two weeks, the grapes are pressed and the juice inoculated with wine yeast to complete the fermentation (Sacchi *et al.* 2005). This method is normally used to produce lighter fruity wines that are meant to be consumed young (Sacchi *et al.* 2005).

Studies done in Spain on Shiraz (Gomez-Miguez *et al.*, 2004) and Tempranillo and Tempranillo/Viura mix (Etaio *et al.*, 2008) have shown that carbonic maceration produce wines that had a low anthocyanin concentration, total phenols, tree fruit and licorice flavours as well as glycerol, titratable acids and colour density but had higher concentrations of red berry aromas.

Most studies have shown that wines made on the carbonic maceration method had low amounts of colour and total phenols (Gomez-Miguez *et al.*, 2004 and Etaio *et al.*, 2008), although some studies show otherwise. Ricardo-da-Saliva *et al.* (1993) tested the effect of skin contact, carbonic maceration and hyperoxidation on Grenache blanc and found that carbonic maceration contained larger concentrations of procyanidins followed by skin contact and hyperoxidation (Ricardo-da-Saliva *et al.* 1993).

#### **2.4.3.3 ENZYMES**

An important factor in the extraction of proanthocyanidins and anthocyanins is the use of enzymes. There are different enzymes in the market for the winemaker to choose from. Every enzyme preparation has a different composition that will have a different mode of working in the juice or wine.

Arnous and Meyer (2009) found that the phenolic composition of grape skins are released by the random liberation of phenols from the grape skin cell wall matrix in a response to progressive enzyme catalyzed degradation of the cell wall polysaccharides. Flavonols and hydrobenzoic acids have been integrated in the skin cell wall polysaccharide via hydrophobic interactions and/or hydrogen bonds (Arnous & Meyer, 2009). Tannins form complexes with cell wall components and are therefore more difficult to release than anthocyanins. With the help of the enzyme components of hemicellulase and cellulase the bond between the tannin complexes are broken and the tannins can be released (Bautista-Ortin *et al.*, 2005). They also found that the flavonol rutin were changed into quercetin as a result of a two step deglycosylation of rutin (Arnous & Meyer, 2009).

The enzymes for anthocyanin extractability appears to be affected by the density of the cell wall polysaccharide matrix, therefore the cell wall act as a protective shield for extraction of the anthocyanins and the anthocyanins are released by cell wall rupturing and not totally by enzymatic activity (Arnous & Meyer, 2009). An extended enzymatic treatment affected anthocyanin negatively as there was a loss of total anthocyanins caused by temperature, while the lost of acylated anthocyanin might be of the formation of hydrocyanamic acids (Arnous & Meyer, 2009).

According to Revilla *et al.* (2003) there are basically two large groups of commercially prepared enzymes: the clarifying and the colour extractors (Revilla *et al.*, 2003). The clarifying enzymes are normally pectolytic enzymes that break down the pectin bonding and therefore help to reduce turbidity of the juice. The colour extractors or maceration enzymes consist of a cocktail of enzymes such as pectin lyase (PL), pectin methyl esterase (PE), polygalacturonase (PG), hemicellulase, cellulose and protease with a glucosidase activity (Revilla *et al.*, 2003). These maceration enzymes not only help with the release of anthocyanins but also help with the extraction of tannins (Revilla *et al.*, 2003 and Arnous & Meyer, 2009).

Commercial enzymes are preparations of a mixture of different enzymes each with their own mode of working. Guerin *et al.* (2009) analyzed 41 commercially available enzyme prepartes to evaluate their mode of working and classify them for their technological effects of sedimentation, clarification, pressing, colour extraction, aroma release and filtration (Guerin *et al.*, 2009). The following enzymatic activity were tested: Pectin methyl esterase (PME), pectin lyase (PL), polygalacturonase (PG), endo(1→4)β-D-glucanase, endo(1→4)β-D-galactanase, endo(1→4)β-D-xylanase, cinnamoyl esterase, β-D-glucosidase, β-D-galactosidase, β-D-xylosidase, β-D-apiofuranosidase, α-L-rhamnosidase and α-L-arabinofuranosidase. Table 2.6 shows the potential technological effects of these enzymatic activities.

Wines with higher colour and tannin concentrations tend to form more stable anthocyanins as the wine matures. There was also an increase in the aroma of the wine as well as a better mouth feel (Bautista-Ortin *et al.*, 2005 & Revilla *et al.*, 2003).

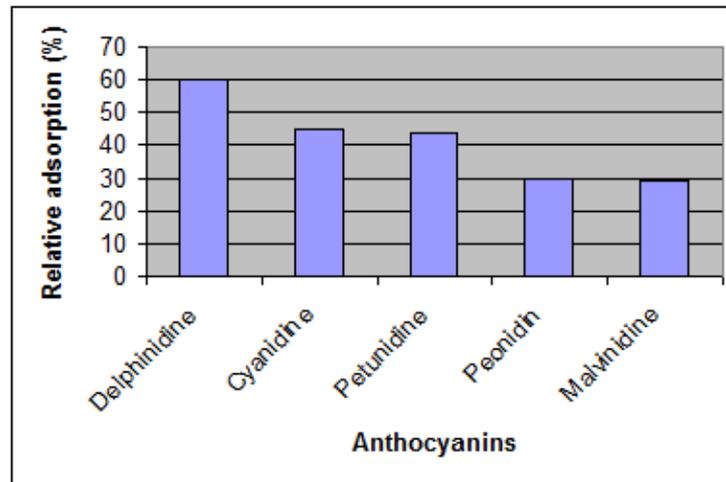
**Table 2.6:** enzymes and their potential technological effects (S-sedimentation, C-clarification, PR-pressing, CE-colour extraction, AR-aroma release, FIL-filtration and \*-indicates effect) (from Guerin *et al.* 2009).

Enzyme	S	C	PR	CE	AR	FIL
Pectin methyl esterase	*	*	*	*		*
Polygalacturonase	*	*	*	*		*
Pectin lyase	*	*	*	*		*
Rhamnogalacturonase		*				*
Galactanase		*				*
Arabinase		*				*
endo(1→4)β-D-glucanase			*	*		
exo(1→4)β-D-glucanase			*	*		
Xylanase			*	*		
Mannanase			*	*		
β-D-glucosidase			*	*	*	
β-D-galactosidase			*	*	*	
α-L-arabinofuranosidase			*	*	*	
α-L-rhamnosidase			*	*	*	
β-D-xylosidase			*	*	*	
β-D-apiofuranosidase					*	
β-Glucanase			*	*		*
Protease		*				*

#### 2.4.3.4 YEAST

It is well known that yeast cell walls had an adsorption effect and that these cell walls may help the winemaker in removing some off-odours as well as proteins. Another effect of yeast cell walls is that it can remove a portion of anthocyanin by adsorption. In a study done by Vasserot *et al.* (1997) they found that it appears that the process of adsorption of anthocyanins by yeast lees is limited by the reach of partition equilibrium between a fraction of anthocyanin that would be free in solution and anthocyanin that would be adsorbed on the yeast cell walls. Scudamore-Smith *et al.* (1990) also found there was a rapid increase in colour density during fermentation but that there was a sharp decrease at the end of fermentation due to adsorption of anthocyanin to yeast lees.

The different anthocyanins have different degrees of polarity and will therefore adsorb differently on yeast lees as can be seen in fig. 2.11. Delphinidin, with the highest polarity, will be the most adsorbed, while malvidin, with the lowest polarity, will be less adsorbed.



**Figure 2.11:** The effect of anthocyanin polarity on its adsorption by yeast lees (Vasserot *et al.*, 1997).

There are different factors that would affect the adsorption of anthocyanin on yeast lees namely: i) Temperature - Lower temperature affects the adsorption capacity better than higher temperature. ii) Ethanol - The amount of anthocyanin adsorbed decrease with an increase in ethanol concentration. iii) SO<sub>2</sub> - As the SO<sub>2</sub> concentration increases the amount of anthocyanin adsorbed decreases. SO<sub>2</sub> also form a reaction between the flavilium cation (A<sup>+</sup>) to form a colourless chromen-2-sulfonic acid (AHSO<sub>3</sub>). iv) pH - As the pH of the wine increases, the amount of anthocyanin adsorbed decreases. Because of an increase in pH the balance of the flavilium cation shifts to the uncharged carbinol pseudobase (Vasserot *et al.*, 1997).

#### 2.4.3.5 THERMOVINIFICATION

Thermovinification is a process where whole or crushed grapes are heated to a temperature between 60-80°C for 20-30 minutes to promote the diffusion of phenolic and colour compounds from the grape skins (Ribereau-Gayon *et al.*, 2000). The heating damage the hypodermal cell membranes and the anthocyanins are released (Lowe *et al.*, 1976; Sacchi *et al.*, 2005). The coloured juice can now be cooled and pressed or pressed and cooled to fermentation temperature and the juice is handled as for normal white juice (Lowe *et al.*, 1976).

Different studies on thermovinification found that the concentration of anthocyanins increases (Baiano *et al.*, 2009, Lowe *et al.*, 1976; Sacchi *et al.*, 2005), while the concentration of the flavonoids remained low (Baiano *et al.*, 2009; Sacchi *et al.*, 2005). In one study, Wagener (1981) found that the wine made from systems (Imeca and

Sernagiotto plants) where they used a destemmer and final press was very dark in colour and astringent (Wagener, 1981). It was also found that the heating of the must denature polyphenol oxidase (PPO) thus preventing browning of the wine (Sacchi *et al.*, 2005).

#### **2.4.3.6 EXTENDED MACERATION**

The rationale behind maceration is that with cold soaking more colour will be extracted from the grape skins and with extended maceration more phenols will be extracted which will help to stabilize wine colour and tannin structure. A study was done on the consequences of extended maceration on red wine colour and phenolic by the University of Adelaide (Unpublished report). They found that extended maceration resulted in lower colour intensity but higher hue (brownier colour) and total phenols than the other treatments. This confirmed a study done by Zimmer *et al.* (2000) where they tested Cabernet Sauvignon from different Californian regions. An extended maceration of twenty days after fermentation showed that the proanthocyanidins significantly increased from 17% to 41% (Zimmer *et al.*, 2002). There was a difference in the phenolic composition of the Pinot noir wine from one week to three weeks of extended maceration. The extra two weeks altered the phenolic composition of the Pinot noir significantly (Unpublished report).

### **2.5 ANALYSIS OF GRAPE AND WINE TANNINS AND ANTHOCYANINS**

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Tannins are also important as they contribute to organoleptic characteristics such as astringency (Monagas *et al.*, 2005; Gawel, 1998), bitterness (Monagas *et al.*, 2005) as well as to colour stability (Monagas *et al.*, 2005), maturation potential (Lorenzo *et al.*, 2005) and health benefits (Corder, 2007). It is for these reasons that the quantification of tannins is very important to the winemaker and viticulturists. For the winemaker to produce a good red wine with a good maturation potential, the tannin and anthocyanin content of that grapes must be known.

There are a large number of different methods to analyze tannins or total phenols in grapes and wine. This section will touch on the different methods. At the end of this section the two most popular tannin methods will also be discussed.

## 2.5.1 DETERMINATION OF TOTAL POLYPHENOLS AND TANNINS

Total polyphenols is a term used to describe compounds which has a benzene ring with one or several hydroxyl groups (-OH) attached to it (Schofield *et al.*, 2001). These polyphenols are then further divided in non-flavonoid (cinnamic acids, caffeic acids etc.) and flavonoids (flavanols, flavonols, flavan-3-ols etc.). The non-flavonoid compounds are mainly found in the pulp of the grape berry and the flavonoid compounds in the skins (proanthocyanidins) and the seeds (procyanidins) of the grape berry (Schofield *et al.*, 2001).

There are many publications available on tannin analyses (Folin and Ciocalteu, 1927, Hagerman and Butler, 1978, Butler *et al.*, 1982, Makkar 1989, Souquet *et al.*, 1996, Ginger-Chavez *et al.*, 1997, Price and Butler, 1997, Sun *et al.*, 1998; Schofield *et al.*, 2001, Hagerman, 2002, Harbertson *et al.*, 2003, De Beer *et al.* 2004, Moris and Silber, 2006, Sarneckis *et al.*, 2006; Seddon and Downey 2008). However, not all of these analyses are applicable to grape tannins. Based on the available literature, the methods for the analysis of grape tannins can be divided into four basic groups, namely colorimetric methods, gravimetric methods, precipitation methods and HPLC (High Performance Liquid Chromatography).

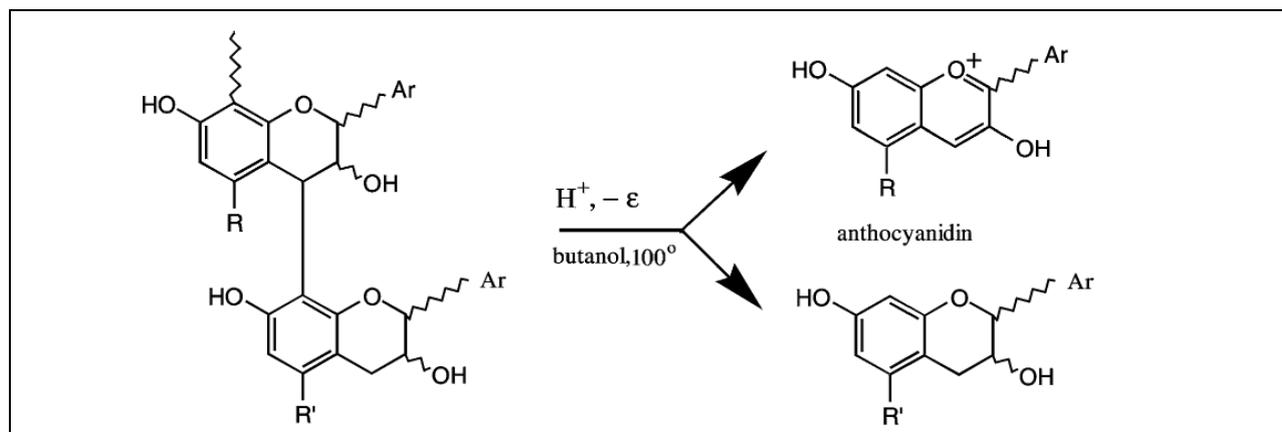
### 2.5.1.1 COLORIMETRIC METHODS

These methods have their basis on the coloring of the solution with either acid-butanol or a dye-like Prussian blue (Price and Butler, 1997). Condensed tannins or proanthocyanidins are mainly found in the skins of grapes (Monagas *et al.*, 2005). They are called proanthocyanidins because they break down to coloured cyanidins and delphinidins in an acid medium (Monagas *et al.*, 2005; Porter, 1986).

#### 2.5.1.1.1 Acid-butanol assay

In the **acid-butanol assay** proanthocyanidins can be quantified (Makkar, 1989). The interflavan links of the condensed tannin are broken under acid conditions and the corresponding anthocyanidins are forced into the colour form which can be determined

at  $A_{550}$  on a spectrophotometer. There are a number of limitations to this assay, as summarized in Schofield *et al.* (2001), which must be taken into consideration.



**Figure 2.12:** The chemistry of an acid-butanol reaction (Schofield *et al.*, 2001)

Firstly, the amount of water in the reaction medium is critical in the colour formation and the quantitative determination of the proanthocyanidins. Secondly, the ease with which the interflavan bonds cleave differs. The  $4 \rightarrow 6$  bonds are more resistant to this cleavage than the  $4 \rightarrow 8$  bonds. Thirdly, the number of phenolic groups in rings A and B affects the wavelength of the absorbance maximum. Fourthly, the colour yield is not always linear with the amount of tannin input. Fifthly, the presence of transitional metal ions in the assay medium is also important. Finally the ratio of acid-butanol:sample medium in the reaction mixture is important (Schofield *et al.*, 2001).

### 2.5.1.1.2 Thioacidolysis

Another form of an acid cleavage reaction is the **thioacidolysis** (thiolysis) or **phloroglucinol**. The mean degree of polymerization is determined with this method as well as (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-O-gallate and their corresponding benzylthioethers (Souquet *et al.*, 1996). With this method condensed tannin is heated with toluene- $\alpha$ -thiol (benzyl mercaptan), which releases the terminal unit as a flavan-3-ol, while the extension units are released as toluene- $\alpha$ -thiol derivatives. The disadvantage of the use of toluene- $\alpha$ -thiol is the strong mercaptan aroma, therefore, a more popular reagent to use is phloroglucinol. The principles for phloroglucinol are the same as for thioacidolysis. These methods are used in conjunction with reverse-phase HPLC to determine the mean degree of polymerization.

### 2.5.1.1.3 Vanillin assay

The **vanillin assay** is used to determine the quantitation of condensed tannin as well as to determine the degree of polymerization (mDP) of proanthocyanidins. The vanillin assay depends on the reaction between vanillin and the condensed tannin to achieve a coloured complex which can be determined at  $A_{500}$ . Because of the concentration of flavan-3-ol end groups the chromophore concentration increases (Butler *et al.*, 1982). This assay is insensitive to the differences between procyanidins and prodelphinidins and therefore catechin is used as the standard, although it can lead to an overestimation of tannin (Butler *et al.*, 1982).

This is a relatively easy assay to use and also relatively quick as it takes about 30 minutes to complete, but there are a number of critical factors which have to be kept in mind. Firstly, the type of solvent used must be considered as at the same normality HCl contains more water than  $H_2SO_4$  and therefore gives a low reproducibility (Sun *et al.*, 1998). The reaction time must be kept at 15 minutes. Temperature must be between 25°C and 30°C. Vanillin concentration must be between 10 – 12 g/L. Finally, the type of reference standard used must be taken into account because purified oligomeric proanthocyanidins expresses the proanthocyanidins content more correctly than (+)-catechin (Sun *et al.*, 1998; Hagerman, 2002).

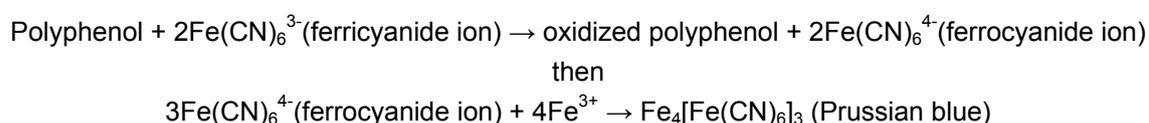
### 2.5.1.1.4 Folin-Ciocalteu method

The **Folin-Denis method** was first described by Folin and Denis (1912) but was later modified to the **Folin-Ciocalteu method** by Otto Folin and Vintila Ciocalteu (1927). Total phenols of wine are determined with the Folin-Ciocalteu assay. This method is based on the reducing power of the phenol hydroxyl group. This method is not very specific and can detect all the phenols (Makkar, 1989) in varying degrees of sensitivity (Sun *et al.*, 1998). Normally, 1 mL of wine are put in a 100 mL erlenmeyer flask with 60 mL of distilled water and 5 mL of Folin-Ciocalteu reagents. After a waiting period of between 30 seconds to 480 seconds, 15 mL of sodium bicarbonate are mixed in the erlenmeyer flask and then topped up to its mark. This is an easy to do assay but it has a long waiting period of about two hours. A standard curve must be set up by using gallic acid as the reference standard. Singleton *et al.* (1974) found that it was difficult to

correlate results from Folin-Ciocalteu assays with HPLC measurements due to the diverse group that constitutes total phenolics in wine.

#### 2.5.1.1.5 Prussian blue assay

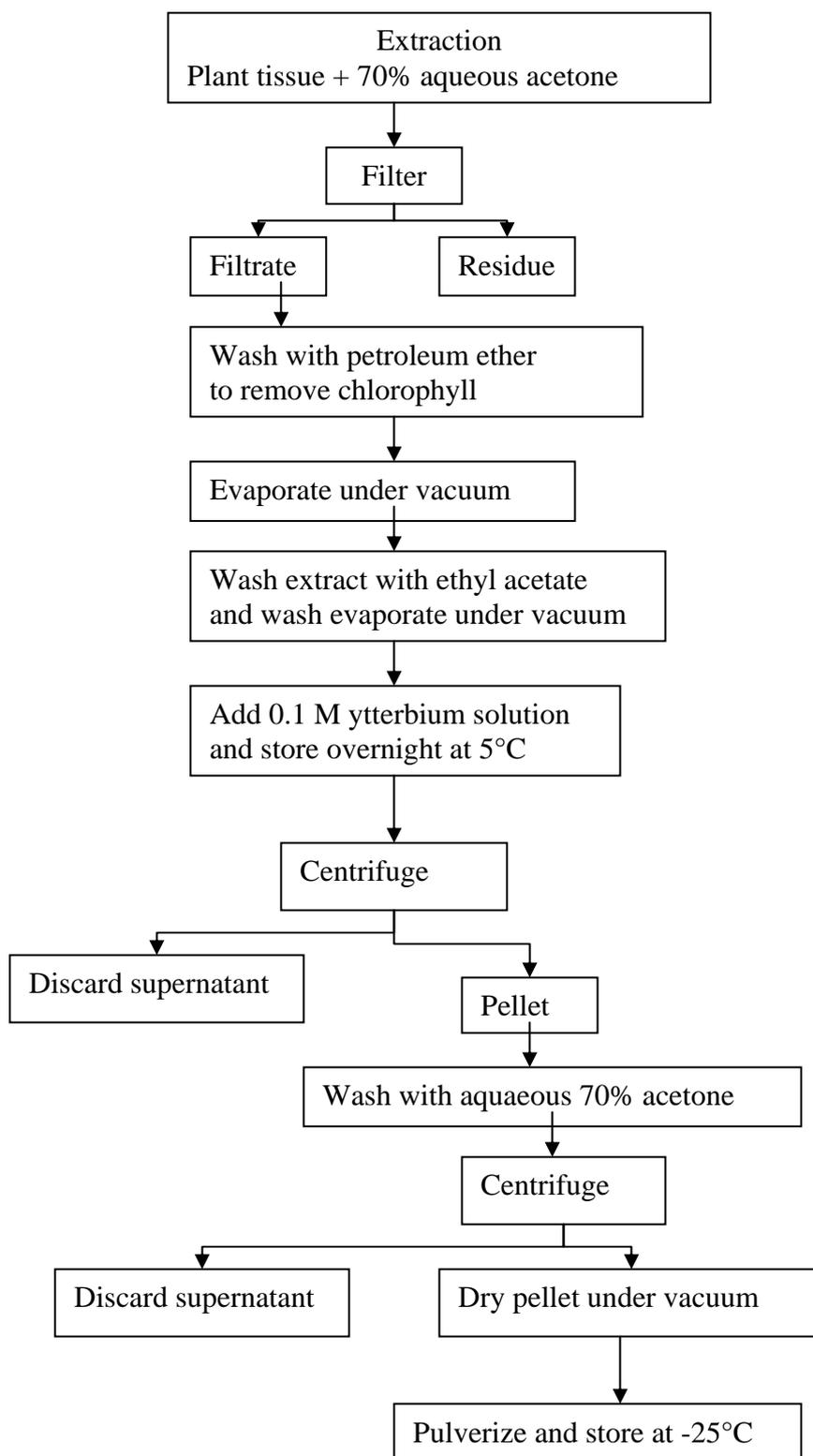
The **Prussian blue assay** is a very simple and quick method with low interference by non-phenolic compounds. This assay was first described by Price and Butler (1977) and it is a popular method for quantification of total phenols (Makkar, 1989). Although this is an easy method it has its shortcomings. There is a formation of a precipitate after a short incubation period and an increase in colour density over a time (Schofield *et al.*, 2001). A summary of the oxidation-reduction reaction looks as follows:



Moris and Silber (February 2006) developed a method where they use **alkaline coomassie brilliant blue combined with Bovine Serum Albumin (CBB-BSA)** to bind to grape tannin. The resulting coloured form is read at an absorbance of  $A_{602}$ . This method worked so well that they developed a hand-held device which can be used in the vineyards to determine the tannin content (Moris & Silber, 2006).

#### 2.5.1.2 GRAVIMETRIC METHODS

The gravimetric methods are very complex methods consisting of a number of steps. However, this method doesn't require a standard for external calibration. This method uses the trivalent cation of a rare earth metal, ytterbium ( $\text{Yb}^{3+}$ ), to form a complex with the tannin and to precipitate it. This precipitate (ytterbium oxide-tannin) is then ashed and weighed. This is compared to an internal and external standard. The external standard consists of cyanidins, delphinidin and purified Quebracho tannin. The internal standard consists of condensed tannins from the plant species that were isolated either with Sephadex LH-20 or trivalent ytterbium (Ginger-Chavez *et al.*, 1997). Figure 2.13 shows the different steps that are used in this method for the determination of total phenols.



**Figure 2.13:** Extraction and isolation procedure of plant condensed tannin using trivalent ytterbium (Ginger-Chavez *et al.*, 1997).

Herderich and Smith (2005) found that the ytterbium method is not suitable for grape tannin analysis of a 50% ethanol solution of grape extract. Ginger-Chavez *et al.* (1997) found that when external standards were used over or under estimation of the tannin

content occurred, however when they used the type of tannin of the plant to be analyzed good results was obtained.

### 2.5.1.3 PRECIPITATION METHODS

According to the definition by White (1957), tannin is a compound that has the ability to precipitate proteins. It is this unique ability of tannin that Hagerman and Butler (1978) used to develop a method to analyze condensed tannins.

#### 2.5.1.3.1 Bovine Serum Albumin (BSA) assay

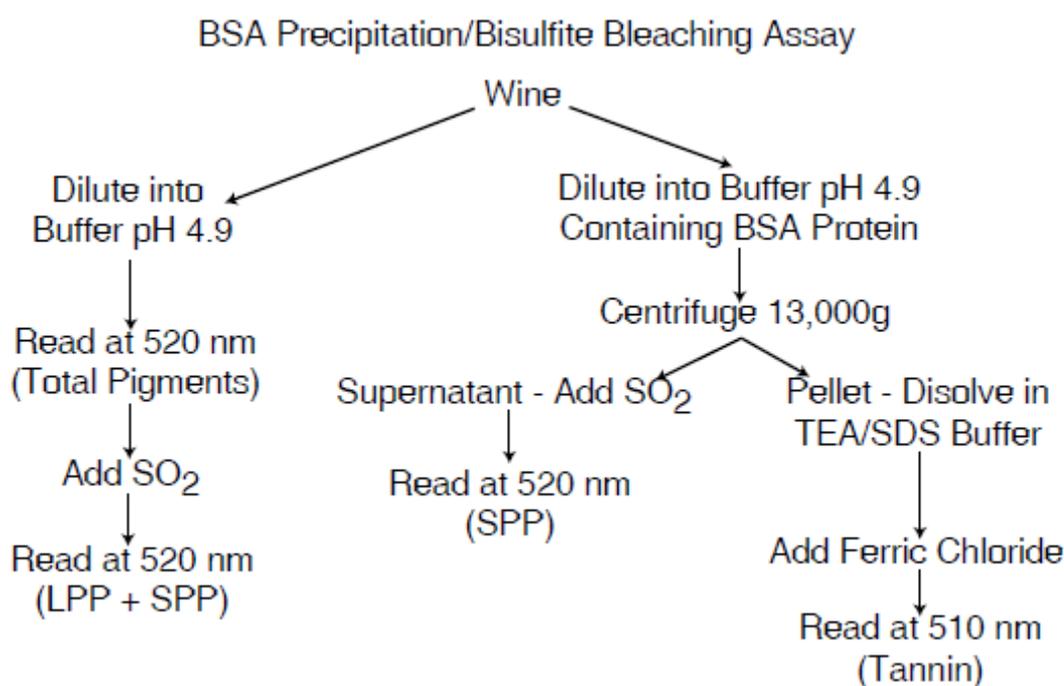
The first of these precipitation methods is the **BSA assay** which was developed in California by Hagerman and Butler (1978). The method involves the formation of protein-tannin complexes (Hagerman and Butler, 1978). Tannin, in a mildly acidic medium, has a negative charge. Protein, on the other hand, has a positive charge. These opposite charges attract each other forming a complex. This complex precipitates in a pellet form after centrifuging of the sample. Hagerman and Butler (1978) decided on Bovine Serum albumin (BSA) as their protein of choice after experimenting with different protein sources (Hagerman and Butler, 1978). The BSA is mixed with an acetate buffer before addition to the wine sample. The mixture is left for 15 minutes at room temperature before it is centrifuged for 15 minutes at 5000 g (Hagerman and Butler, 1978) or for 5 minutes at 13000 g (Harbertson *et al.*, 2003). The pellet, that is formed, is redissolved with a 1% sodium dodecyl sulfate (SDS) and 5% triethanolamine (TEA) buffer. In the original method developed by Hagerman and Butler (1978) they used 1% SDS, but in the study done by Harbertson *et al.* (2003) they used 5% of SDS. After another 10 minutes at room temperature the mixture is measured in a spectrophotometer at  $A_{510}$  as  $T_{\text{background}}$ . The mixture is then mixed with ferric chloride and is then also read at  $A_{510}$  as  $T_{\text{final}}$  after standing 10 minutes at room temperature. By using the following formula:

$$\text{Eq.1} \quad \text{Tan} = (T_{\text{final}} - T_{\text{noise}}) - (T_{\text{background}} * 0.875)$$

For  $T_{\text{noise}}$  Buffer C (SDS and TEA) and Ferric chloride is mixed and measured at  $A_{510}$  on the spectrophotometer. The answer of the above mentioned equation is put into a standard curve to get to the final tannin concentration of (+)-catechin equivalents (CE)

in mg/L. With the addition of the ferric chloride the mixture turns blue. This method is an indirect method for tannin analysis and the result is actually ferric binding tannin.

Harbertson (2003) modified the BSA assay by combining it with a bisulfate bleaching step. This is done at three different steps as can be seen in figure 2.14 and the result is the determination of monomeric pigments (MP), small polymeric pigments (SPP) and large polymeric pigments (LPP). With the first step one can differentiate between monomeric anthocyanins from the polymeric pigments. With the other two steps one can differentiate between the small polymeric pigments (SPP), which does not precipitate, and the large polymeric pigments (LPP), which does precipitate.



**Figure 2.14:** Schematic representation of the determination of tannin and polymeric pigments in grape extracts and wine by protein precipitation combined with bisulfate bleaching (Harbertson *et al.*, 2003).

Table 2.7 shows the LPP, SPP, LPP + SPP and the tannin concentrations from Cabernet Sauvignon wine with the addition of 400 µg and 800 µg of tannins.

**TABLE 2.7:** Absorbance (520 nm) in a Cabernet Sauvignon wine due to LPP, SPP and total polymeric pigments (LPP+SPP). Tannin was measured at the same time using the assay in figure 5 (Harbertson *et al.*, 2003).

	LPP <sup>a</sup>	SPP <sup>a</sup>	LPP+SPP <sup>b</sup>	Tannin
Wine	1.47	1.03	2.50	1107
Wine+400µg tannin	1.58	1.00	2.58	1515
Wine+800µg tannin	1.62	1.01	2.63	1900

<sup>a</sup> Absorbance (520 nm) due to LPP or SPP calculated for undiluted wine.

<sup>b</sup> mg/L catechin equivalents.

### 2.5.1.3.2 Methyl Cellulose precipitation (MCP) method

The second precipitation method is called the **methyl cellulose precipitation assay (MCPT)** which was developed in Australia (Sarneckis *et al.*, 2006). According to this method the polysaccharide methyl cellulose, which has also a positive charge, will bind to tannin. After centrifugation of the mixture a pellet is formed. Two microfuge tubes are prepared – one with the methyl cellulose and one without. Both of the microfuges are measured in a spectrophotometer at an absorbance of A<sub>280</sub> (UV spectrum) in a quartz cuvette. The two values are subtracted from each other. The final value is put into the standard curve to get to the final tannin concentration which is in (-)-epicatechin equivalents (EE mg/L).

### 2.5.1.4 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is a very accurate method for the identification and quantification of monomeric to trimeric proanthocyanidins, although it is difficult to differentiate between polymeric tannins and complex compounds found in wine. This can be done by using two variations on the HPLC method, namely normal-phase and reverse-phase HPLC.

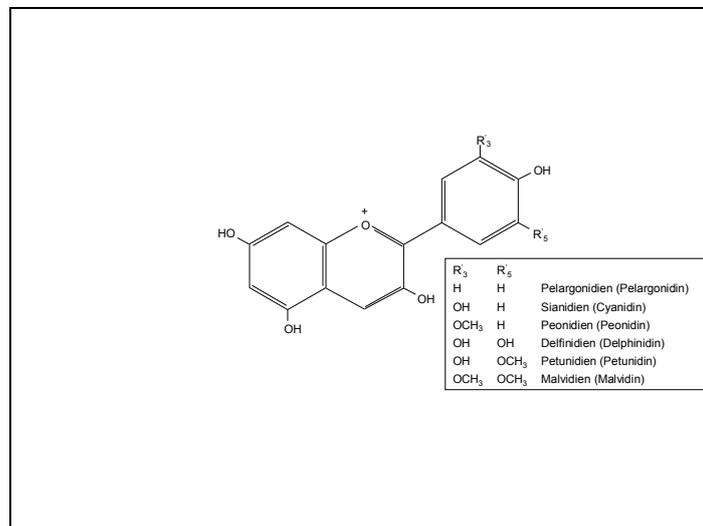
**Normal-phase HPLC** uses polar packing with a non-polar mobile phase, which means that polar compounds (hydrophilic compounds) will elute first while the non-polar compounds (hydrophobic compounds) will elute last. In a study done by De Beer *et al.* (2004) the proanthocyanidins, which were detected at 280 nm, were divided in three groups: monomers, low molecular-weight proanthocyanidins and high molecular-weight proanthocyanidins.

**Reverse-phase HPLC** uses non-polar packing with a polar mobile phase. This means that the non-polar compounds (hydrophobic compounds) will elute first while the polar compounds (hydrophilic compounds) will elute last. In the same study done by de Beer *et al.* (2004) the compounds that were identified were benzoic acids, hydrocinnamates, flavan-3-ols, flavonols and anthocyanins. De Beer *et al.* (2004) found that (-)-epicatechin co-elute with anthocyanins and could therefore not be successfully quantified.

According to Sarneckis *et al.* (2006) there is a very good correlation between the tannin measurements by the MCP assay and the reverse-phase HPLC. Of the 121 Australian red wines that was analyzed the correlation was  $R^2 = 0.74$  and from the 54 grape extracts it was  $R^2 = 0.79$ . According to a study done by Seddon and Downey (2008) they found just the opposite. They found that there is a poor correlation between the MCP and BSA assays ( $R^2 = 0.41$ ), and that the correlation between the protein precipitation and the HPLC-phloroglucinolysis is very good ( $R^2 = 0.91$ ), while the correlation between the methyl cellulose and the HPLC-phloroglucinolysis is very poor ( $R^2 = 0.25$ ).

## 2.5.2 ANTHOCYANINS AND THE DIFFERENT METHODS FOR ANTHOCYANIN ANALYSES

Anthocyanin is very important for red wine's colour. Anthocyanin is a flavyllium cation with a positive charge. When a glucose molecule is glycosylated to this flavyllium cation an anthocyanin is formed. As shown in figure 2.15 there are at least five different anthocyanins in wine, which range from pink to a deep purple red.



**Figure 2.15:** Different forms of anthocyanins

Anthocyanins are found mainly in the vacuoles of the epidermal cells. There are a few methods to determine the anthocyanin content of grapes, namely Iland method and wine, namely HPLC, colour density, hue, Boulton and Levensgood, Somers and Evens and Ribéreau-Gayon and Stonestreet.

### 2.5.2.1 Iland method

Patrick Iland (2000) devised a method to determine the total anthocyanin in grapes. The skin of red and black grapes is coloured various shades of red and/or bluish black due to the presence of red coloured pigments, mainly anthocyanins. The anthocyanins are extracted from the skins during fermentation. They, along with oligomeric and polymeric compounds give red wine its colour.

A measure of the concentration of red coloured pigments in berries can give an indication of the potential colour of wine made from those grapes. The relationship between the measure of grape colour and wine colour is based on the assumption that: i) all the anthocyanins are extracted from the skins, ii) there is no loss of anthocyanins due to precipitation or polymer formation and iii) all wines are made in a similar manner.

The method is based on the extraction, with ethanol, of the pigments from a known weight of macerated whole grapes. 100 Berries are weighed and homogenized in a homogenizer. A portion of 2 mg of the homogenized grapes are extracted in an ethanol solution (pH 2.0). This extract is mixed on a rotator (100 rpm) for an hour before it is centrifuged. The extract is then diluted with 1 M HCl and left for three hours after which the absorbance of this solution measured on a spectrophotometer at 520 nm. The calculation of red pigments is based on the use of the extinction coefficient of malvidin-3-glucoside and the result is expressed as equivalents of this anthocyanin.

**Eq. 2** mg of anthocyanin/berry =  $(A_{520_{HCl}}/500) \times 11 \times (21 \text{ mL}/100) \times (\text{weight of 100 berries (g)}/\text{weight of homogenate (g)}) \times (1000/50)$

**Eq. 3** mg of anthocyanin/berry =  $(A_{520_{HCl}}/500) \times 11 \times (21 \text{ mL}/100) \times (\text{weight of 100 berries (g)}/\text{weight of homogenate (g)}) \times (1000/\text{weight of homogenate (g)})$

Studies have shown that the measure of grape colour can act as a predictor of wine colour. Generally, in practice the measure of grape colour can predict if a wine will be lightly, moderately or intensely red coloured. It can also in some cases provide an indication of the flavour intensity of the wine and wine quality, particularly when grapes and wines produced from within a similar climatic region is compared.

### 2.5.2.2 Colour density

**Colour density** is probably the easiest and quickest method to get an indication of the anthocyanin content. This method gives the value in absorbance units (AU). The traditional method (Glories, 1984) to determine colour intensity is by calculating the sum of yellow/brown, red and purple hues absorbance as shown in Eq 4.

**Eq. 4**                      **Colour density =  $A_{420} + A_{520} + A_{620}$**

Patrick Island (2005) divided wines based on their colour density in three categories:

- AU < 6 = light wines
- AU 6-10 = medium wines
- AU > 10 = heavy wines

Therefore, colour density measures how much colour there is in a wine (Harbertson, 2006). Low colour densities of a wine from the same region compared to wine with high colour density can be explained by the pigment composition and the state of pigment equilibrium of that wine (Somers and Evans, 1974). Factors that can affect colour density are the pH and the sulphur dioxide (SO<sub>2</sub>) content of the wine (Somers and Evans, 1974). It was found (Somers and Evans, 1974) that 20 to 25% of the total anthocyanins are in the coloured flavyllium form at pH 3.4 to 3.6. Sulphur dioxide (SO<sub>2</sub>) in wine exists in the bisulphate (HSO<sub>3</sub><sup>-</sup>) form. This HSO<sub>3</sub><sup>-</sup> has a decolouring effect on the wine as the HSO<sub>3</sub><sup>-</sup> binds to the flavyllium ion to form a colourless flavene sulphonate (Somers and Evans, 1974). As the anthocyanins polymerize into polymeric pigments they become less sensitive to SO<sub>2</sub> and pH changes (Somers and Evans, 1974).

To determine the **SO<sub>2</sub>-resistant anthocyanins** or **anthocyanin colour**, some of the wine sample is bleached with a 20% sodium metabisulphate solution. The difference of the bleached sample and the wine colour gives the anthocyanin colour (Somers and Evans, 1974).

**Eq. 5**                      **Anthocyanin colour =  $A_{520} - A_{520}^{SO_2}$**

**Eq. 6**                      **Polymeric pigment colour =  $A_{520}^{SO_2}$**

The **tone or hue** of a wine is used to monitor wine ageing (Harbertson, 2006). This is calculated as the ratio between  $A_{420}$  and  $A_{520}$ .

**Eq. 7**                      **Hue/tone =  $[A_{420}/A_{520}]$**

In young wines these values are between 0.5 – 0.7 and reach values of 1.2 – 1.3 in aged wines. This is a very useful way to look at ageing of red wine as the shift from red to brick red (Harbertson, 2006).

### **2.5.2.3 Boulton and Levenson assay (copigment assay)**

A factor that traditional analytical methods didn't consider is the tendency of young red wines to form self-associations or copigmentations due to the molecular associations between anthocyanins and other organic molecules in the solution. It is apparent that copigmentation account for between 30 and 50% of the colour of young wines. It is these associations that cause the anthocyanins to exhibit a far greater colour than was expected (Boulton, 2001).

Levenson and Boulton (2004) used spectrophotometric measures to determine the copigmentation of Cabernet Sauvignon. This is a time-consuming method that can take as long as 75 minutes to 90 minutes as it involves different steps. First of all the pH of the wine must be adjusted to pH 3.6 and filtered with a 0.45  $\mu\text{m}$  filter. Acetaldehyde is put in an aliquot with the wine and the colour is determined at 520 nm after a 45 minute wait. Wine is diluted in another aliquot and the colour is also determined at 520 nm after 10 minutes.  $\text{SO}_2$  solution is added in a third aliquot and the colour is also determined after 10 minutes at 520 nm. The following formula is then used to determine the colour due to copigmentations.

**Eq. 8**                      **Colour due to copigmentation =  $A_{\text{acet}} - A_{\text{dilute}}$**

Levenson and Boulton (2004) found that the mean absorbance unit (AU) for colour due to copigmentation fall in a range of 1.81 – 5.67 (mean of 3.34), while the AU for anthocyanins was in a range of 1.20 – 3.23 (mean of 2.01).

In a comparison study done by Versari *et al.* (2008), an experiment was done to determine the correlation between Boulton's copigmentation method and reverse-phase HPLC. The analysis of the phenolic compounds was done by reverse-phase (RP-C<sub>18</sub>) column. Versari *et al.* (2008) found an overall good correlation with total colour ( $R^2 =$

0.8999), free anthocyanins ( $R^2 = 0.9159$ ) and a very good correlation with copigmentation ( $R^2 = 0.9464$ ).

#### 2.5.2.4 Somers and Evans assay

This is a long method where the anthocyanins are all forced into the red flavyllium form by mixing the wine sample with 1N HCl. The mixture is left for 3 -4 hours after which the total anthocyanin content is determined at 520 nm. Sodium metabisulphate is mixed with wine and also determined at 520 nm. The anthocyanin concentration is then expressed as mg/L (Rivas-Gonzalo *et al.*, 1992).

$$\text{Eq. 9} \quad \text{Anthocyanin concentration (mg/L)} = 19.6*(A_{520}^{\text{HCl}} - A_{520}^{\text{SO}_2})$$

#### 2.5.2.5 Ribéreau-Gayon and Stonestreet assay

This is a faster method than Somers and Evans's method as it take about 15 minutes. One mL of wine is mixed with 1 mL ethanol/HCl solution (96% ethanol + 0.1 v/v HCl) and 20 mL of an aqueous solution. In two test tubes is alliquitted 10 mL of the above mixture. In one test tube 4 mL of water is added and in the other test tube 4 mL NaHSO<sub>3</sub>. After 15 minutes the samples from both test tubes are measured at 520 nm (Rivas-Gonzalo *et al.*, 1992).

$$\text{Eq. 10} \quad \text{Anthocyanin concentration (mg/L)} = 615*(A_1 - A_2)$$

A study done by Rivas-Gonzalo *et al.* (1992) showed that the values for the Ribéreau-Gayon & Stonestreet and Somers & Evans methods were always higher than those obtained by HPLC. Table 2.8 shows the values that Rivas-Conzalo *et al.* obtained from their studies that show the higher values from the methods of Ribéreau-Gayon & Stonestreet and Somers & Evans. Their reasoning was that HPLC measures only free anthocyanins while the other two methods also evaluate part of the polymers which is sensitive to the effect of pH and bisulphate bleaching.

**TABLE 2.8** Contents in anthocyanins (mg/L) determined by different methods in sample of a wine, taken along the vinification process (Rivas-Gonzalo *et al.*, 1992).

Day	AC RS	AC RSm	AC SE	AC SEm	AC HPLC
7	697	490	527	516	363
10	670	471	507	497	354
15	590	415	481	472	288
25	547	384	439	430	201
60	499	351	389	381	203
85	476	334	403	395	209
115	489	343	388	380	204
150	480	338	370	363	192
200	472	331	325	318	175
260	445	312	338	331	177
345	343	240	242	238	153

AC RS – anthocyanin from the method of Ribereau-Gayon and Stonestreet, AC RSm – Ribereau-Gayon and Stonestreet's method modified, AC SE - anthocyanin from the method of Somers and Evans, Ac Sem – Somers and Evans' method modified.

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# **Chapter 3**

## **Research results**

**The influence of different winemaking techniques on the extraction of grape tannins and anthocyanins from Cabernet Sauvignon, and Shiraz grapes**

This manuscript will be submitted for publication in the  
**South African Journal of Enology and Viticulture**

## RESEARCH RESULTS

### 3.1 ABSTRACT

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The aim of this study was to determine the effect of different maceration techniques on the extraction of grape tannins and anthocyanins. Two cultivars (Cabernet Sauvignon and Shiraz) were harvested in two different climatic regions (Durbanville and Simondium) at two different ripeness levels. Five basic winemaking processes were applied, namely a normal alcoholic fermentation (C), enzyme treatment (E), cold maceration (CM), post maceration (PM) and a combination of cold and post maceration (CM+PM). At harvest the phenolic ripeness was determined with the Glories method, while the tannin concentration was determined with the methyl cellulose (MCP) and bovine serum albumin (BSA) methods. It was found that the tannin precipitation methods (MCP & BSA) have shown the same tendency. The warmer area had higher tannin levels than grapes harvested in the cooler area in both years. In the 2009 harvest season the enzyme treatment showed a better tannin extraction. CM+PM showed the best effect with early ripeness (Cabernet Sauvignon) and CM with fuller ripeness in the warm area. CM showed the best results with either early or fuller ripeness levels in cooler area. PM showed the best effect with the early ripeness levels & E with the fuller ripeness levels in the warm area. CM+PM showed the best results with early ripeness level in cooler area & varied results with the fuller ripeness levels. In both years the cooler area had more anthocyanin than the warmer area. In 2008 (both the early and fuller ripeness levels) & 2009 (early ripeness levels) CM showed the best result in anthocyanin extraction. At a fuller ripeness level (2009) the treatments had no effect.

### 3.2 INTRODUCTION

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The fact that red wine contains tannin and anthocyanin is well known. The importance of tannin is its contribution to organoleptic characteristics such as astringency (Monagas *et al.*, 2005; Gawel, 1998), bitterness (Monagas *et al.*, 2005), colour stability (Monagas *et al.*, 2005), maturation potential (Lorenzo *et al.*, 2005) and also to health benefits (Corder, 2007). Anthocyanin on the other hand, is responsible for the colour of red wines.

The Webster's Dictionary (1989) states that the word **macerate** originates from the French word *maceratus* which means to soften by steeping in a liquid, with or without

heat. Thus maceration is a technique that is used to extract components and pigments out of grape pomace by leaving the skins on the juice or wine for an extended time before or after fermentation.

There are certain factors that can influence maceration: temperature, contact between solids and liquids, degree of agitation, contact time and composition of the extracting liquid (Robinson, 1999). The concentration of anthocyanins and tannins can be influenced by manipulating the abovementioned factors. Different techniques have been developed to help the winemaker in achieving their goal for colour and tannin extraction. In a popular article in the *Wines & Vines* (June 2009) Tim Patterson wrote that the idea of cold maceration was invented in Burgundy by a controversial Lebanese winemaker called Guy Accad in the 1980's for the heartbreak grape –Pinot noir. Pinot noir is known for the fact that it doesn't have high concentration of colour and other methods must be used to increase the colour intensity. This maceration has divided the winemaking community into two groups. The one group believes in maceration while the other group maintains that normal fermentation will have the same effect. According to McMahon (1999) the rationale behind cold soaking is that the aqueous extraction in a non-alcoholic matrix improves wine colour and colour stability and may increase aromatic intensity. However, the research on the effect of maceration is limited.

Temperature or heat is a means of degrading grape tissue. It increases the break up of grape tissue components and accelerates maceration (Ribereau-Gayon *et al.*, 2000). Alcoholic fermentation causes an increase in the temperature of the grape must and therefore the extraction of grape tissue components are increased during this period. Furthermore, the increase in ethanol that occurs during alcoholic fermentation also increases extraction (Robinson, 1999).

Contact between skins, seeds and the juice have an impact on colour and tannin concentration. A study done by Ferré (1953) showed that at least 80% of the grape colour is extracted on the sixth day of maceration.

The degree of agitation is part of a great deal of research. Two types of agitation can be identified. The first method is the so-called punch down of the cap (Fr. *Pigeage*). The grapes were firstly stomped with the feet, but as the vats/tanks got bigger and deeper and the danger of toxic carbon dioxide gas grew bigger, other methods were used. The

caps were broken up by wooden punches, but as this is a very labour intensive job, pneumatic punches were invented. Now there are pneumatic punches which can be set to punch down every couple of hours for a certain amount of time. Spin-offs of these methods are the rotofermenters and the autovinificators (Robinson, 1999). The second method is the pump over of the wine/juice over the cap (Fr. *Remontage*). Here the juice/wine is drawn from the bottom of the tank and are then pumped over the cap. The force can be used to break up the cap, or the juice/wine can be sprayed onto the cap so that the juice/wine seeped through the cap. If the pump overs are done at the end of fermentation more seed tannins are extracted and the seed tannins are needed with the skin tannins to give a balanced wine (Ribereau-Gayon et al., 2000).

Maceration of grapes can be divided in two groups. After crushing of the grapes the vacuoles of the skin cells are broken and the anthocyanins can seep out. The absence of alcohol, at that time, allows the formation of higher molecular weight pigmented phenolic species which will enhance colour stability (McMahon *et al.*, 1999). This can be done at different temperatures and for different lengths of time as a number of studies have shown. Gomez-Plaza *et al.* (2000) used Monastrell to be cooled down to 10°C for 5 days and in 2001 tested three different maceration times (4, 5 and 10 days). In both studies Gomez-Plaza *et al.* (2000) found that the low maceration temperature (10°) and longer maceration time (10 days) had a positive influence on the anthocyanin concentration, chromatic characteristics and hydroxyl cinnamic acid derivatives of the wines. After storage of twelve months the wines made with the longer maceration time (10 days) kept their colour density and phenolic contents longer than the shorter maceration times (4 days) (Gomez-Plaza *et al.*, 2000). Longer skin contact time may lead to greater polymerization of pigments and higher concentrations of procyanidins and therefore to a greater colour stability in the early stages of red wines (Gomez-Plaza *et al.*, 2001).

Post maceration where the skins are left in the wine after fermentation are completed for up to two/three weeks (Joscelyne & Ford, 2008 and Zimmer *et al.*, 2002). This maceration will extract more seed tannins and care should be taken not to use this method if the cultivar is prone to too high concentration of tannins. The rationale behind maceration is that with cold soaking more colour will be extracted from the grape skins and with extended maceration more phenols will be extracted which will help to stabilize wine colour and tannin structure (Joscelyne & Ford, 2008). A study was done on the

consequences of extended maceration on red wine colour and phenolic by the University of Adelaide (Joscelyne & Ford, 2008). They found that extended maceration resulted in lower colour intensity but higher hue (brownier colour) and total phenols than the other treatments (Joscelyne & Ford, 2008).

The aim of this study is to compare the two tannin precipitating methods (BSA & MCP) with each other and to determine the impact of different maceration techniques have on the extraction of tannins and anthocyanins. The difference between early (before commercial harvest) and later (after commercial harvest) ripeness levels are compared to the extraction of tannin and anthocyanin concentrations. The effect of phenolic ripeness is also compared to the different harvesting times.

### 3.3 MATERIALS AND METHODS

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#### 3.3.1 ORIGIN OF GRAPES

In 2008 and 2009 two cultivars of *Vitis vinifera*: Cabernet Sauvignon and Shiraz were used in this trial from two different climatic areas. The first area is classified as IV according to the Winkler scale (1965) i.e. a warm area. This area is in the Stellenbosch district of Simondium on the farm Plaisir de Merle. The second area is classified as III according to the Winkler scale (1965), therefore it is regarded as slightly cooler than the first area. This second area is a located in the Durbanville district on the farm Morgenster. The Winkler heat summation works as follows: The sum of the average daily temperature above 10°C for the growth months (September to March) are calculated and are then expressed as degree days. These degree days are then compared to a table. This table can then be used to calculate which cultivar would suite the specific area.

**Table 3.1** Winkler heat summation adapted for South African climate

Area	Degree days	Potential for viticulture
I	<1389	Early cultivars, high quality, no mass production
II	1389 – 1667	High quality white and red table wine
III	1668 – 1944	Late cultivars, high quality red
IV	1945 – 2222	Natural sweet cultivars, medium quality red & white
V	>2222	Mass production, late cultivars, dessert wines

The vineyards of Plaisir de Merle: the Cabernet Sauvignon is grafted on Richter 99 while the Shiraz is grafted on Mgt 101-14. The row direction for the Shiraz are South-East to North-West, while the Cabernet Sauvignon is South to North. The vineyards are thus planted to be exposed to the morning sun. The vineyards of Morgenster: the Cabernet Sauvignon and Shiraz is grafted on Richter 99. The row direction for the Cabernet Sauvignon is in an East-West direction, while the Shiraz is in a South-West – North-East direction and therefore the sun moves over the rows.

During the 2008 and 2009 seasons grapes were harvested at two different ripeness levels. The low ripeness levels were harvested at an early stage before commercial harvest and the higher ripeness level after commercial harvest.

### **3.3.2 HARVESTING AND WINEMAKING**

An average of 200 kg grapes were harvested for each cultivar at both locations and for both ripeness levels. The grapes were destemmed and crushed into a bin. The SO<sub>2</sub> concentrations were lifted to 25 parts per million (ppm). The grape must and skins were separated, weighed and then divided into twelve homogeneous samples containing equal amounts of skins and juice.

The grape musts were subjected to four winemaking processes namely a control, cold maceration, post maceration and a combination of cold and post maceration (no variation on these processes were looked at) to follow the extraction of tannin. The control treatment (C) was inoculated with 25 g/hL WE372 (Anchor, South Africa) directly after destemming and were pressed just after alcoholic fermentation was completed. All the other treatments were also inoculated with 25 g/hL WE372. During the cold maceration (CM) treatments the must was left on the skins for three days at 15°C before the must was inoculated (Addendum B for the wine analysis). After alcoholic fermentation the wine was pressed. During the post maceration (PM) treatment, the must was inoculated after destemming, but after alcoholic fermentation the skins was left on the wine for two weeks at room temperature before pressing (Addendum B for wine analysis). With the combination of cold and post maceration (CM+PM) treatments the must was left on the skins for three days at 15°C before inoculation and after alcoholic fermentation the skins was left on the wine for two weeks at room temperature before pressing. The grapes were pressed through three times a day. After alcoholic

fermentation the total SO<sub>2</sub> of every wine was adjusted to 50 mg/L. Before bottling the total SO<sub>2</sub> was again adjusted to 50 mg/L. Each treatment was done in triplicate.

The experiment was repeated in 2009, but another treatment was added, an enzyme (E) treatment. The enzyme treatment was handled precisely the same as for the control but with an enzyme addition before the start of fermentation. The pectolytic enzyme (0.04 mL/L) that was used was Pectinex Ultra SPL from Novozym SA (South Africa).

### **3.3.3 SAMPLING OF GRAPES AND CHEMICAL ANALYSIS**

Five hundred berries were randomly selected from the twelve crates of which 100 berries were weighed and homogenized. The homogenization was done in a Retch GM200 GRINDOMIX for 30 seconds at 5000 rpm. The remaining grapes were sent to a contracted laboratory to be analyzed for phenolic ripeness. Two grams of the homogenate were used for colour analysis and one gram for tannin analysis.

Samples of the wine were taken at different stages in the winemaking process for tannin and anthocyanin concentrations analyses. These stages are after cold maceration, after alcoholic fermentation, after post maceration, after bottling, and 3 and 6 months after bottling.

### **3.3.4 TANNIN ANALYSIS**

The samples were prepared for tannin analysis by mixing 1g homogenized grape must with a 10% Aqua solution (50% deionized water and 50% of 96.5% ethanol). This mixture was shaken for an hour on a rotator. The samples were then centrifuged in a Beckman Model J2-21 centrifuge for ten minutes at 4500 rpm. The supernatant were used for tannin analysis, using the BSA assay as described by Hagerman and Butler (1978) and later modified by Harbertson (2003) and the MCPT assay as described by Sarneckis *et al.* (2006). The analyses took place after alcoholic fermentation (AF), after bottling (BOT) at three months maturation (3MD). Every sample was done in triplicate.

The percentage of tannin extracted from the grapes to the wine was calculated as follows:

$$\frac{\text{Mg/L tannin in the wine}}{\text{Mg/L tannin in the grapes}}$$

### **3.3.5 ANTHOCYANIN ANALYSIS FOR GRAPES AND WINE**

Anthocyanin analyses of grapes were done according to the method of Iland *et al.* (1993). For the total anthocyanin analysis of wine, a 100 µL of wine were mixed with 10 mL of 1 M of HCl, shaken and left to stand for three hours before the sample were read at 520 nm on a spectrophotometer. The analyses took place after alcoholic fermentation (AF), after bottling (BOT) at three months maturation (3MD). Every sample was done in triplicate.

### **3.3.6 ANALYSIS FOR COLOUR DENSITY AND HUE**

Colour density and hue were also measured with a spectrophotometer. Colour density of the wine was measured in a 2 mm quartz cuvette at 420 nm and 520 nm. These two absorbance spectrums were added together to obtain the colour density. For colour hue the 420 nm value were divided by the 520 nm value. The analyses took place after alcoholic fermentation (AF), after bottling (BOT) at three months maturation (3MD). Every sample was done in triplicate.

### **3.3.7 ANALYSIS OF PHENOLIC RIPENESS**

The analysis of phenolic ripeness using the Glories method (1984) was outsourced to two contract laboratories.

### **3.3.8 STATISTICAL ANALYSIS**

Data was analyzed using STATISTICA version 9 (Tulsa, U.S.A). A mixed model repeated measures ANOVA and post-hoc tests were conducted using Fisher LSD (least significant difference) model. The error bars were set as 95% confidence intervals and on the supposition that the variances are the same, therefore the error bars will have the same width.

### 3.4 RESULTS AND DISCUSSION

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Several methods are available to the farmer/viticulturist/winemaker to establish the ripeness level of grapes. Methods like the traditional °Brix, pH and TA, °Brix:pH, TA:pH, °Brix:TA or °Brix x (pH)<sup>2</sup> (du Plessis and van Rooyen, 1982) can be used, but they all have limited success. Erika Winter (2004) compiled a book on Berry Sensory Assessment which describes the different methods that the viticulturist can use to determine the ripeness of the grapes. Although these are not foolproof methods, they certainly give indications to the farmer/viticulturist/winemaker as to the potential quality of the grapes and, of course, the ripeness as well. The phenolic ripeness of grapes was a topic that was of great interest to Glories (1984). In 1984 he developed a method to determine the phenolic ripeness of grapes by looking at the extractability of anthocyanin in the wine.

#### 3.4.1 PHENOLIC RIPENESS ACCORDING TO THE METHOD OF GLORIES

The effect of grape ripeness level on tannin extractability is illustrated by the results in Table 3.2 and 3.3. Cabernet Sauvignon from Plaisir de Merle had an EA% of 55 when the grapes were harvested at 22.7°B, but when the grapes reached 27.1°B the EA% dropped to 37. The Shiraz from Plaisir de Merle reached phenolic ripeness at 24°B as the extractability of the grapes did not improve at the higher sugar level. The reason for the increase in EA% was due to a decrease in total anthocyanin while the extractable anthocyanin basically stayed the same. The Cabernet Sauvignon grapes from Morgenster also reached phenolic ripeness at 23.5°B where the EA% was 38. The Shiraz from Morgenster in 2009 shows a low extractability (59 to 54%). The reason for these higher values was that grapes were not fully ripe when the grapes were harvested as Morgenster is situated in a cool area which prolongs ripening.

The percentage monomeric pigments (MP%) is the contribution of the seed tannins to the wine and therefore the higher the MP% value the more astringent the wine will be. As the berry ripens the MP% decreased. This was especially prominent with the cultivars on Plaisir de Merle of both harvest seasons and the same tendency could be seen for Morgenster. The Shiraz on Morgenster was heavily infected with *Botrytis cinerea* and it could explain the low MP% values for the 2009 harvest season. On both

of these farms the MP% decreases as the berries ripens, but the MP% of the Shiraz on Morgenster was very low in 2009.

Phenolic maturity is reached when the concentration of grape anthocyanins is at its maximum (Ortega-Regules *et al.*, 2006). In most cases, except for the Cabernet Sauvignon on Morgenster from the 2009 harvest, the total anthocyanin probably reached their peaks at the early ripeness levels as the total anthocyanin decreases with time. According to Ortega-Regules *et al.* (2006) these grapes have reached their phenolic maturity. This also correlates with Glories extractability, which was below the 40% cut-off point for phenolic ripeness of the 2008 harvest season, but not for the 2009 harvest season.

**Table 3.2** Phenolic ripeness analysis according to the Glories method of the grapes harvested during the 2008 and 2009 seasons at Plaisir de Merle

Analyses	Plaisir de Merle							
	Cabernet Sauvignon				Shiraz			
	2008		2009		2008		2009	
	26/2	28/3	6/2	2/3	4/3	28/3	18/2	2/3
°B	22.7	27.1	20.7	23.8	24.0	27.0	23.4	24.6
BM (g)	1.49	1.30	1.25	1.18	1.88	1.27	1.20	1.43
TA	991	713	1432	1286	848	817	925	765
EA%	55	37	44	46	38	35	33	42
MP%	58	52	41	33	60	54	51	39
T (mg/L)	6690	7360	4970	6350	5720	6520	3960	4430

LB – low balling, HB – higher balling, BM – berry mass, TA – Total Anthocyanin (mg/L), EA% - percentage extractable anthocyanins, MP% - percentage monopigments and °B – degrees balling at harvest and T – tannins in mg/L catechin equivalents.

**Table 3.3** Phenolic ripeness analysis according to the Glories method of the grapes harvested during the 2008 and 2009 seasons at Morgenster

Analyses	Morgenster							
	Cabernet Sauvignon				Shiraz			
	2008		2009		2008		2009	
	4/3	4/4	12/3	14/4	23/2	5/3	24/3	14/4
°B	21.4	23.3	21.1	23.3	20.5	24.4	23.0	24.4
BM (g)	1.52	1.31	1.61	1.60	1.48	-	1.70	1.62
TA	1141	778	913	999	1387	-	1463	1440
EA%	43	38	42	39	43	-	59	54
MP%	49	52	38	36	41	-	16	12
T (mg/L)	5400	5730	4290	6350	5610	5770	4410	3960

LB – low balling, HB – higher balling, BM – berry mass, TA – Total Anthocyanin (mg/L), EA% - percentage extractable anthocyanins, MP% - percentage monopigments and °B – degrees balling at harvest and T – tannins in mg/L catechin equivalents. No analysis was done on the Shiraz of Morgenster in 2008 because of Botrytis infection.

### 3.4.2 COMPARING THE TWO TANNIN PRECIPITATION METHODS

Two of the most popular methods for tannin analysis are the bovine serum albumin (BSA) and methyl cellulose precipitable (MCP) methods. There are a few factors to consider when using these methods. These factors include repeatability, time efficiency and ease of practice. These two methods were compared against each of these factors.

The average difference between the tannin concentration of BSA and MCP assay is 1:4.5 (data not shown). Therefore the tannin concentration according the BSA assay is much lower than with the MCP assay. If the tannin concentration in a wine is naturally low, it would be difficult to get a reading from the BSA assay.

*Comparrison between the BSA and MCP methods:* It was found that the correlation between the BSA and MCP method was very good ( $R^2 = 0.88$ ), while Seddon and Downey (2008) found the oppisite where the correlation between BSA and MCP was poor ( $R^2 = 0.41$ ). While both methods precipitate tannins, they do not precipitate the same amount of tannins and in all likelihood, do not precipitate the same type of tannins or subclasses of tannins. This may be due to differences in the ability of BSA versus MCP to bind and precipitate tannins with different structural features or differences in the affinity of the BSA and MCP for each tannin subclass (Seddon and Downey 2008). The correlations between the BSA and the HPLC ( $R^2 = 0.28$ ) and MCP and the HPLC ( $R^2 = 0.32$ ) was very poor. A reason for the poor correlation between the BSA and MCP methods and the HPLC method could be because the standards for the HPLC did not include polymeric tannins and that the HPLC was standardized with only monomers and dimers (B1 & B2). Sarneckis *et al.* (2006) found that the correlation between the MCP and reverse-phase HPLC was good ( $R^2 = 0.74$ ). Seddon and Downey (2008) also found that the correlation between the BSA and reverse-phase HPLC was very good ( $R^2 = 0.91$ ), while the correlation between the MCP and reverse-phase HPLC was very poor ( $R^2 = 0.25$ ).

Every sample was done in triplicate. The tannin for the triplicate was worked out and an average was taken. From this average the standard deviasion (stdev) was worked out. The CV% is obtained by deviding the stdev in the average. The standard deviation of all the samples tested, for the MCP method, was on average 86 mg/L, while the CV% was 1.9%-2.1%. The standard deviation for all the samples tested, for the BSA method, was

on average 3 mg/L, while the CV% was 1.5%-3.6%. Both of these methods compared good in repeatability while the MCP method is a much easier method to use. Harbertson *et al.* (2008) analyzed 1325 red wine samples made of the cultivars Cabernet Sauvignon, Merlot, Pinot noir and Zinfandel taken from the USA, Australia and France with the BSA method. They found that the standard deviation was at least half of the mean concentration and that within a single cultivar the variation was 32-fold. In another study done by Brooks *et al.* (2008), they found that there was a big variation between tannin concentrations of the same sample. So they took three bottles of wine and sent it to five different laboratories which do the BSA method. They found that the CV% of the laboratories was 27%. They concluded that the BSA method was not repeatable and that the tannin concentration could be much higher than anticipated.

*Time efficiency* is a property that must be considered when analyzing for tannin concentration, especially in situations where a high sample through-put is required.

There are about 3 times as many steps in the BSA method as in the MCP method. This increases the risk for increasing variation. In our analyses it took 90 minutes for six samples with the BSA method but it took only 45 minutes for the same six samples with the MCP method. Mercurio and Smith (2008) came to the same conclusion. It took 45 minutes for 48 samples with the MCP method and 90 minutes for 10 to 15 samples with the BSA method. The BSA method takes longer to do than the MCP method, but with the BSA method the polymeric pigments can also be analyzed. This is a positive attribute if the evolution of polymeric pigments is to be followed.

*The ease of practice:* The MCP method is an easy-to-do method as it has only two steps. The first reason why the BSA method is not an easy method is that MP, SPP and LPP can also be measured which takes much longer to do with more steps included. If the polymeric pigment analyses are not important then these steps can be excluded. The second reason is that the BSA method consists of multiple steps. The third reason is that the BSA method uses five different solutions and for every solution different amounts have to be pipetted into the vessel used. Care must be taken to make sure that every time the same amount of solution must be pipetted otherwise the repeatability will be compromised.

Although there are a lot of discrepancies between the two precipitation methods, the choice of method must be considered by the repeatability of the method, the time efficiency, the ease of practice and what you want to achieve. Jensen *et al.* (2008) also recommended that the samples be diluted so that the tannin response should be between 0.3-0.75 absorbance units under the given circumstances. When comparing one of these precipitation methods to a HPLC, the standardization of the HPLC must be correct. As neither of these methods measure monomers (Harbertson and Downey 2009), the HPLC must be standardize with longer chains of polymers. Harbertson and Downey (2009) also concludes that the nature of the tannins precipitated by the two methods is different and that this must be fully explored.

### **3.4.3 THE EXTRACTION OF TANNIN AND ANTHOCYANIN BY DIFFERENT WINEMAKING PROCESSES.**

It is widely believed that with cold maceration more of the anthocyanins are extracted as anthocyanins are located in the vacuoles of the cells of the grape skins (McMahon *et al.*, 1999, Gomez-Plaza *et al.*, 2000 & 2001, Alvarez *et al.*, 2009 & Gil-Munoz *et al.*, 2009). Tim Patterson (Wines & Vines, 2009) interviewed a couple of American winemakers. He found that the winemakers can be divided in two camps. Some of them believe that cold maceration had a profound effect on anthocyanin extraction, and the other camp believes there is no effect.

Tannin is extracted as soon as alcohol is produced. Tannin is not water soluble and is therefore better extracted in an alcoholic medium (Canals *et al.*, 2005).

#### **3.4.3.1 THE EFFECT ON TANNIN EXTRACTION BY DIFFERENT WINEMAKING PROCESSES**

##### *Grapes*

The grapes of the cultivars Cabernet Sauvignon and Shiraz from both farms were analyzed for tannin concentration according to the MCP (Sarneckis *et al.*, 2006) method. Grapes were harvested at a low ripeness level (before commercial harvest) and at a higher ripeness level (after commercial harvest). The tannin concentration of the grapes was compared to the tannin concentration after alcoholic fermentation (AF). In most cases the percentage of tannin extracted were higher for the fuller ripeness

levels than for the lower ripeness levels (Table 3.4). This is expected as the tannins are more extractable in riper grapes than for greener grapes.

**Table 3.4:** The average tannin extracted from the cultivars Cabernet Sauvignon and Shiraz from both farms measured after alcoholic fermentation of all the treatments from both farms (Plaisir de Merle and Morgenster) of the 2008 and 2009 harvest season. The tannin concentration was measured by the MCP method.

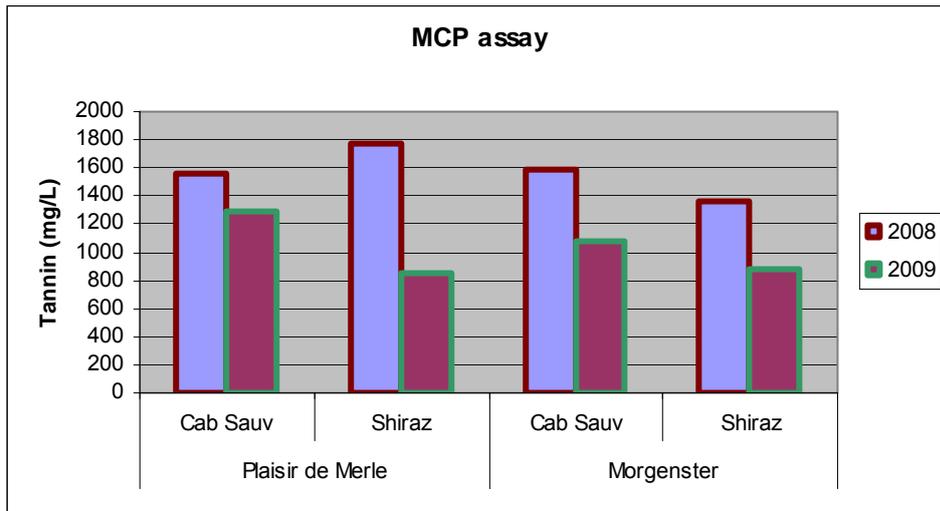
Cultivars	Farm	°B	2008	2009
Cabernet Sauvignon	Plaisir de Merle	22.7	23%	23%
Cabernet Sauvignon	Plaisir de Merle	27.1	23%	27%
Shiraz	Plaisir de Merle	23.4	20%	29%
Shiraz	Plaisir de Merle	27.0	25%	30%
Cabernet Sauvignon	Morgenster	21.5	20%	24%
Cabernet Sauvignon	Morgenster	24.0	22%	30%
Shiraz	Morgenster	20.5	17%	27%
Shiraz	Morgenster	26.4	27%	25%
<b>Total</b>			<b>22%</b>	<b>27%</b>

As can be seen in table 3.4, the average tannin concentration extracted from the grapes into the wine is 22% for 2008 and 27% for 2009. Adams and Scholz (2007) found that between 10%-58% is the norm for extraction. This shows that our extraction falls in the parameters founded by Adams and Scholz.

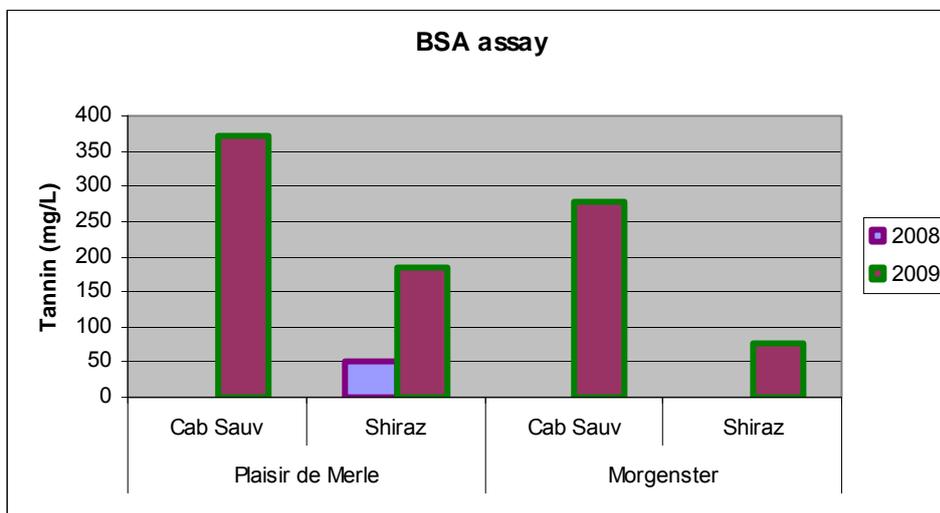
#### *Extraction treatments in winemaking*

Figure 3.1(a) depicts the total tannin of the Cabernet Sauvignon and Shiraz wine for Plaisir de Merle (Winkler scale IV, hereafter the warmer farm) and Morgenster (Winkler scale III, hereafter the cooler farm) for the 2008/9 harvest seasons. Figure 3.1(a) show that the tannin concentration of the two cultivars from the farm located in the warmer area was similar between the two seasons. In general the warmer farm had more tannin concentration than the cooler farm. This applies for both the Cabernet Sauvignon and Shiraz cultivars. The average tannin concentration difference between the wines from the two farms was between 15-20%. This could be explained as tannin formation is influenced by optimal day temperature of 17°C-26°C (Jackson and Lombard, 1993). The gene for the expression of tannin is expressed at optimal temperature of 25°C and is inhibited at temperatures lower than 18°C (Mori *et al.*, 2005 & Chorti *et al.*, 2010).

a)



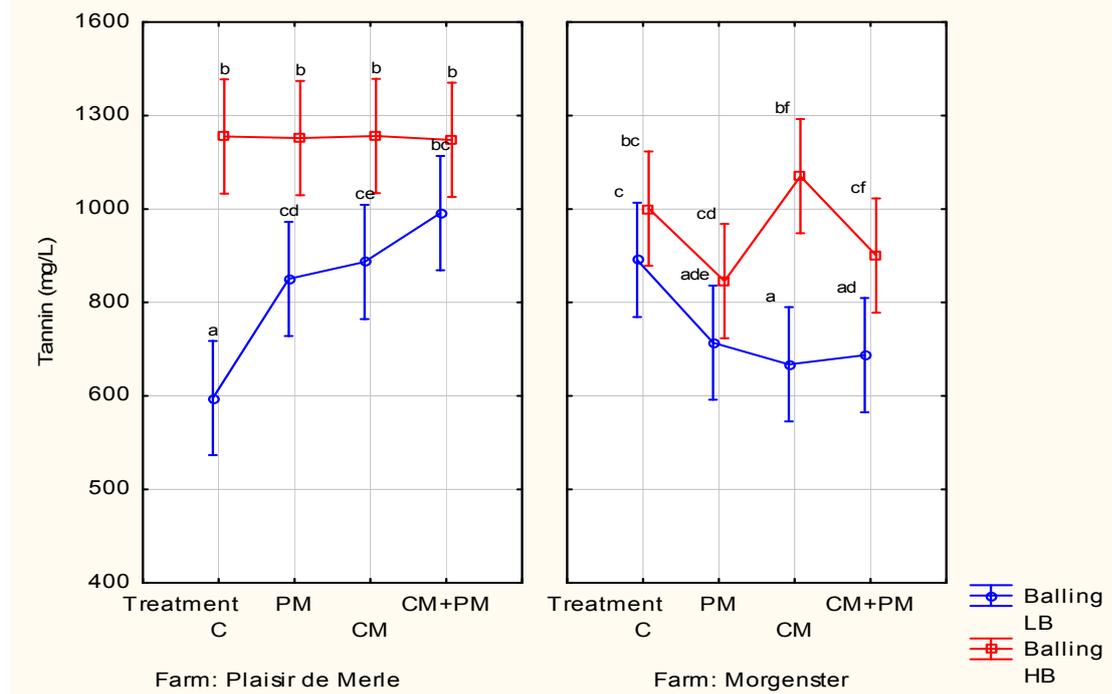
b)



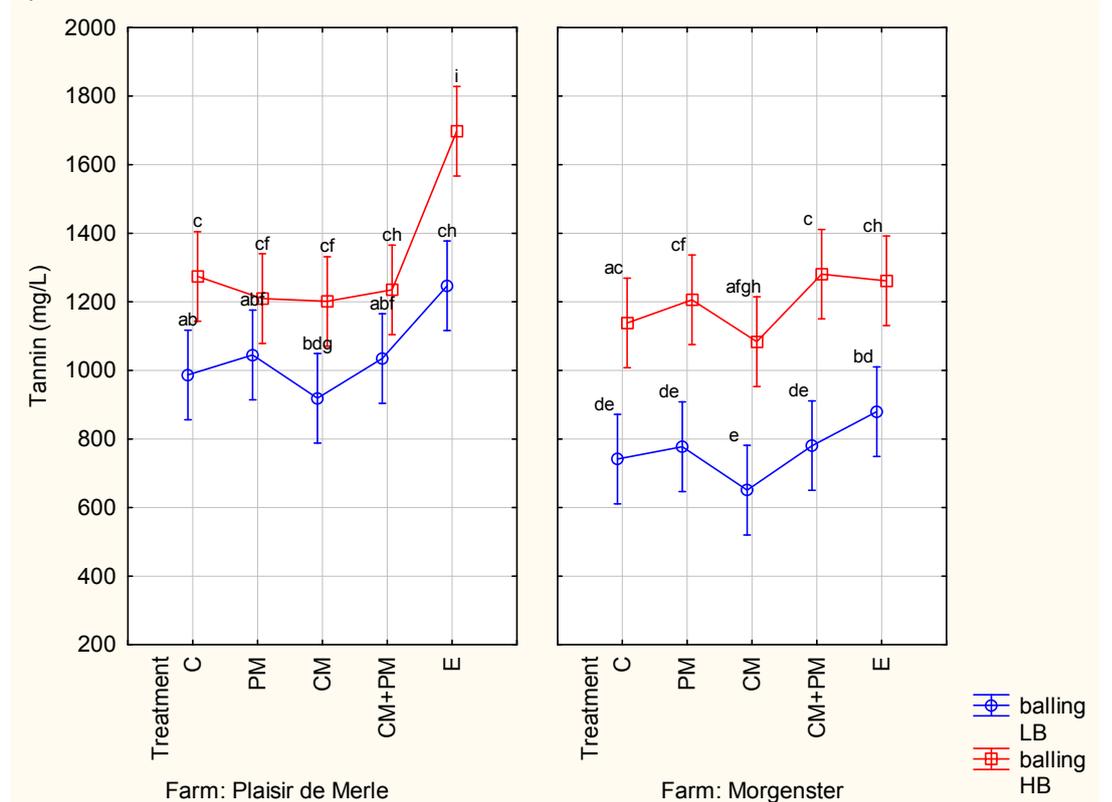
**Figure 3.1:** The average of the total tannin of the wine from Plaisir de Merle and Morgenster for Cabernet Sauvignon and Shiraz from the 2008 and 2009 harvest seasons with a) the MCP assay and b) the BSA assay.

Figure 3.1(b) depicts the total tannin of Cabernet Sauvignon and Shiraz for the warmer farm and the cooler farm for the 2008/9 harvest seasons as measured by the BSA method. The data of the 2008 harvest is not comparable due to problems with the method. In the 2009 harvest season the warmer area showed more tannin concentration than for the cooler area. This trend is the same for both cultivars. The tannin concentration values are between 3.8-4.8 times lower with the BSA compared to the MCP method.

a) 2008



b) 2009



**Figure 3.2:** The average tannin concentration (MCP assay) of Cabernet Sauvignon wines made with the different extraction techniques during the 2008 harvest season of each farm of the 2008 harvest season (a). The treatments are: C – control wine, PM – post maceration, CM – cold maceration, CM+PM – combination of cold and post maceration. In 2008 the Cabernet Sauvignon for LB – 22.7°B for Plaisir de Merle (harvested on 26 February) and 20.5°B for Morgenster (harvested on 4 March), HB – 27.1°B for Plaisir de Merle (harvested on 28 March) and 23.5°B for Morgenster (harvested on 4 April). In the 2009 harvest season the Cabernet Sauvignon for (b) LB – 20.9°B for Plaisir de Merle (harvested on 6 February) and 21.1°B for Morgenster (harvested on 12 March), HB – 23.8°B for Plaisir de Merle (harvested on 2 March) and 23.0°B for Morgenster (harvested on 14 April).

The tannin concentration of the control wine, made from the low balling grapes from the warmer farm of the 2008 harvest [figure 3.2 (a)], is significantly lower than any of the treatments, showing that any one of the different treatments could be used to extract tannin at a lower ripeness level. There is a slight difference between the post and cold maceration, but the difference is not significant. The combination treatment (cold and post maceration) shows a somewhat larger difference although this was also not significant. Although not significantly higher than the other treatments, the combination treatment produced a wine containing the highest tannin as been depicted in figure 3.2.

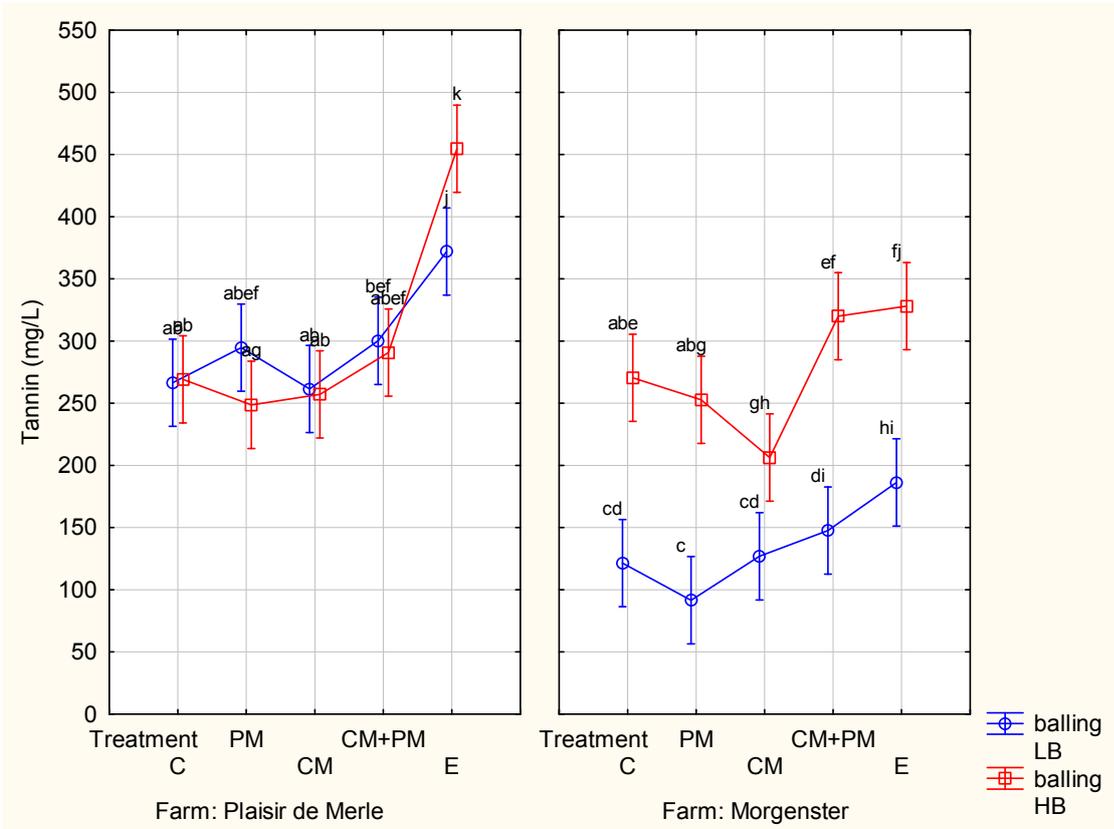
Figure 3.2 shows that there are no differences between the treatments in the warmer area at the higher ripeness level. This probably indicates that at a low ripeness level, the tannin structure is not fully developed and that different treatments can extract more tannin concentration from the grape skins and seeds.

On the cooler farm of the 2008 harvest season [figure 3.2 (a)], the treatments showed a negative effect against the control at the low ripeness level. However, at the higher ripeness level the PM and CM+PM treatments showed a negative effect i.e. lower tannin concentration than for the control. In contrast, the CM treatment showed similar tannin concentrations than the control. The treatment of CM, at the higher ripeness level, show a slightly higher tannin concentration than the C (not significant) wine and significantly higher than the treatment of PM. This trend did not appear in the 2009 harvest season.

In contrast the 2009 data of the warmer farm [figure 3.2 (b)] the low ripeness level show that there were no significant differences between the treatments. The only treatment that showed a significant increase in tannin concentration was the E treatment. This trend was the same for both the ripeness levels from the warmer area.

At the higher ripeness level, the same trend can be seen as in the 2008 harvest season. Again showing those at a higher ripeness levels the different treatments had no effect on tannin extraction. The results suggest that the tannin structure of the grapes is more matured at a higher ripeness level. The only treatment that had any significant effect on tannin extraction on both the low and high ripeness levels was the enzyme treatment. This can also be expected as the enzymes tend to break the bonding between the tannin and the cell wall compounds releasing more tannin into the wine.

In the 2009 harvest season the trend between the low and high ripeness levels are essentially the same, just the absolute concentrations differ. There was no difference between the C wine and the treatments of PM and CM+PM, meaning that longer skin contact didn't improve tannin extraction. The only difference at the low ripeness level was the E treatment, but the E treatment didn't have any effect at the higher ripeness levels. To conclude the treatments on tannin extraction, one can see that at a warmer farm CM, PM and CM+PM treatments will have an effect on tannin extraction at low ripeness levels, but not at higher ripeness levels. Pectolytic enzyme preparates are suggested for increased tannin extraction (Guerin *et al.*, 2009).



**Figure 3.3:** The average tannin concentration (BSA assay) of Cabernet Sauvignon of each farm of the 2009 harvest season. The treatments are: C – control wine, PM – post maceration, CM – cold maceration, CM+PM – combination of cold and post maceration. In 2008 the Cabernet Sauvignon for LB – 22.7°B for Plaisir de Merle (harvested on 26 February) and 20.5°B for Morgenster (harvested on 4 March), HB – 27.1°B for Plaisir de Merle (harvested on 28 March) and 23.5°B for Morgenster (harvested on 4 April).

Figure 3.3 show the tannin concentration as measured by the BSA method. The treatments had no significant effect on tannin concentration on both the ripeness levels from the warmer farm except the E treatment resulted in significant higher concentrations of tannin. There was not much difference in tannin concentration of the ripeness levels from the warmer area. The cooler area shows a large difference in tannin concentrations between the ripeness levels. At the low ripeness level, the

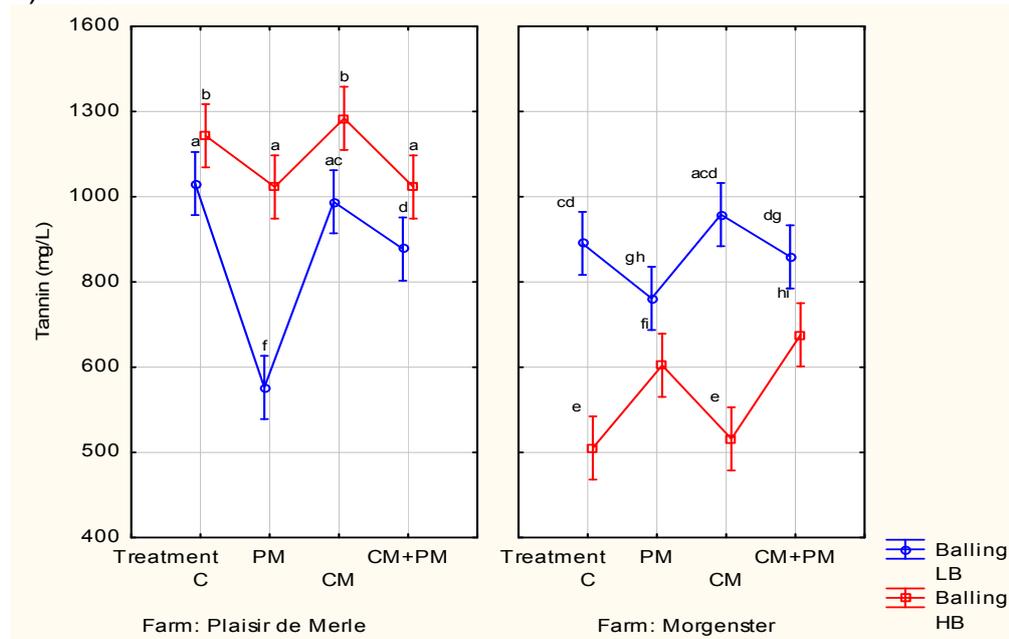
treatments did not show differences on tannin concentration except the E treatment which had a significant effect on tannin concentration. At the higher ripeness the combination treatment of M and the E treatment show significant differences in tannin concentration above the other treatments. It seems that to extract more tannin pectolytic enzymes must be used (Guerin *et al.*, 2009).

At the warmer farm [figure 3.4 (a)], there is a decline in tannin concentration of the PM treatment in the 2008 harvest season. The same slight decline in tannin concentration can be seen with the combined CM+PM treatment. However, this trend is very different from what has been seen for Cabernet Sauvignon. This could mean that there were a lot of monomers in the wine or that polymerization took place and many of the tannins precipitated after PM.

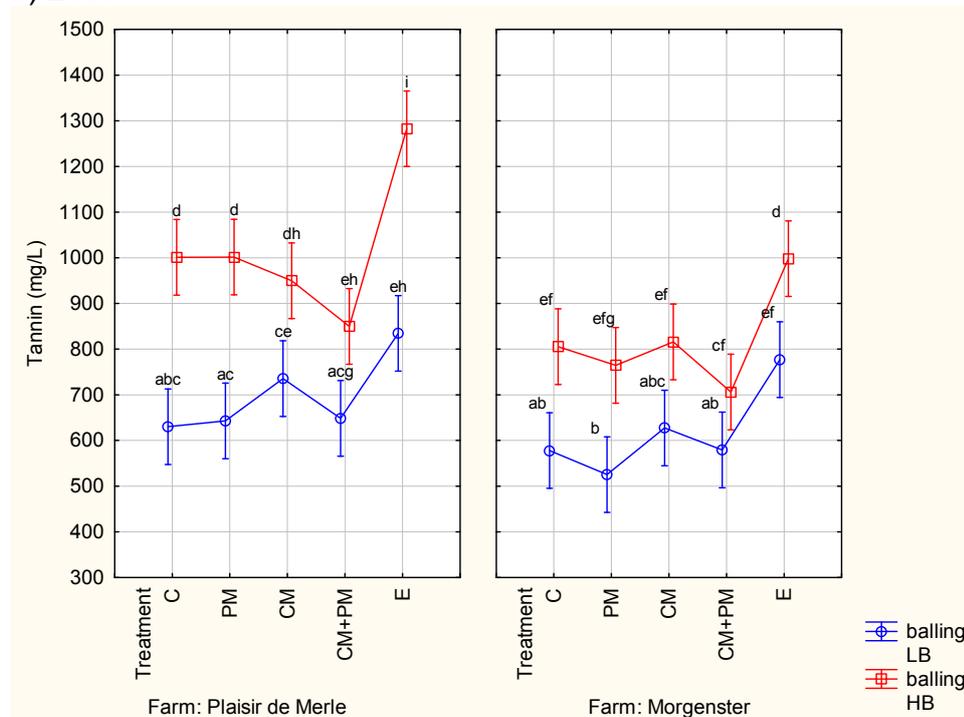
On Morgenster [figure 3.4 (a)] the PM treatment and also the combined CM+PM treatment had a significant effect on tannin concentration at a higher ripeness level, but the opposite was true at a lower ripeness level where the CM treatment had the best effect.

Figure 3.5 shows the tannin concentrations as measured by the BSA method. On the warmer farm, at the higher ripeness level, the CM and CM+PM treatments showed a negative effect resulting in low tannin concentration while the E treatment significantly increases the tannin concentration. At the low ripeness level only the E treatment showed increase in tannin concentration. The same can be seen on the cooler farm where the treatments did not have an effect on tannin concentration, except for the E treatment that showed a significant increase in the tannin concentration. The CM and PM show a negative effect at the higher ripeness level for the warmer area.

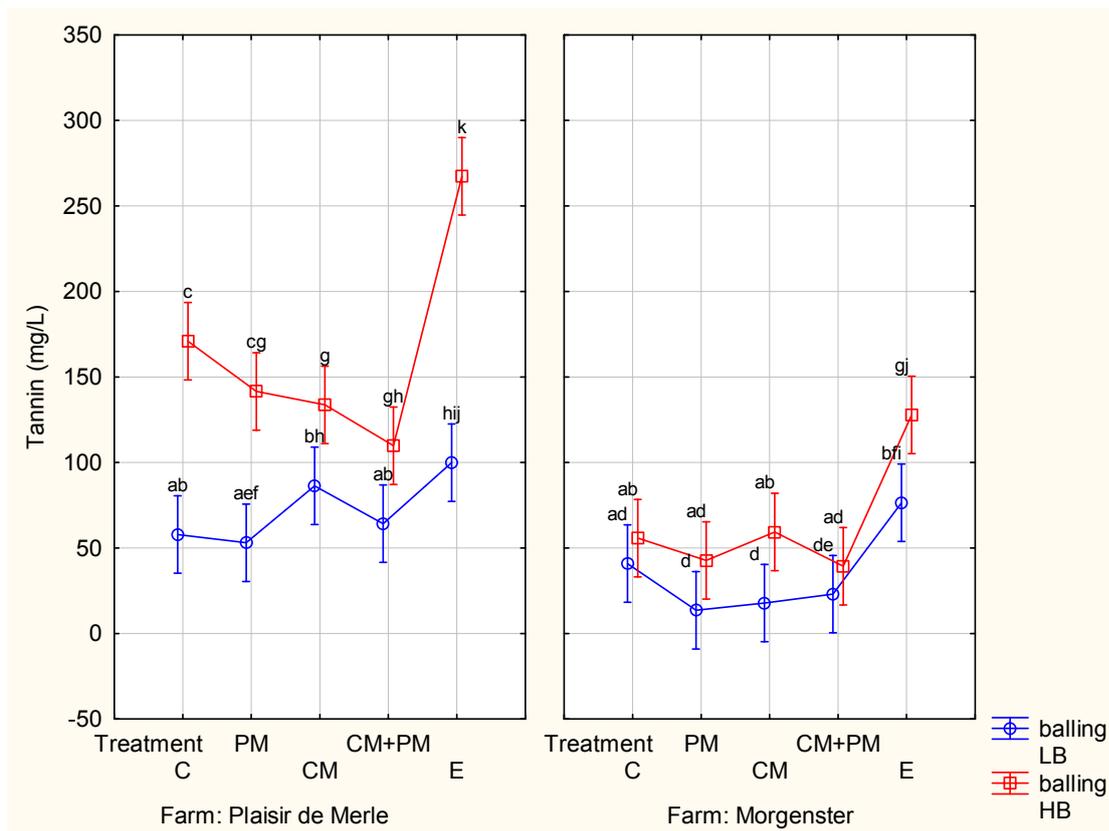
a) 2008



b) 2009



**Figure 3.4:** The average tannin concentration (MCP assay) of Shiraz of each farm of the 2008 harvest season (a). The treatments are: C – control wine, PM – post maceration, CM – cold maceration, CM+PM – combination of cold and post maceration. In 2008 the Shiraz for (a) LB – 24.0°B for Plaisir de Merle (harvested on 4 March) and 20.5°B for Morgenster (harvested on 23 February), HB – 27.0°B for Plaisir de Merle (harvested on 28 March) and 24.4°B for Morgenster (harvested on 5 March). In the 2009 harvest season the Shiraz for (b) LB – 23.4°B for Plaisir de Merle (harvested on 18 February) and 21.8°B for Morgenster (harvested on 24 March), HB – 24.6°B for Plaisir de Merle (harvested on 2 March) and 24.4°B for Morgenster (harvested on 14 April).



**Figure 3.5:** The average tannin concentration (BSA assay) of Shiraz of each farm of the 2009 harvest season. The treatments are: C – control wine, PM – post maceration, CM – cold maceration, CM+PM – combination of cold and post maceration. In the 2009 harvest season the Shiraz for (b) LB – 23.4°B for Plaisir de Merle (harvested on 18 February) and 21.8°B for Morgenster (harvested on 24 March), HB – 24.6°B for Plaisir de Merle (harvested on 2 March) and 24.4°B for Morgenster (harvested on 14 April).

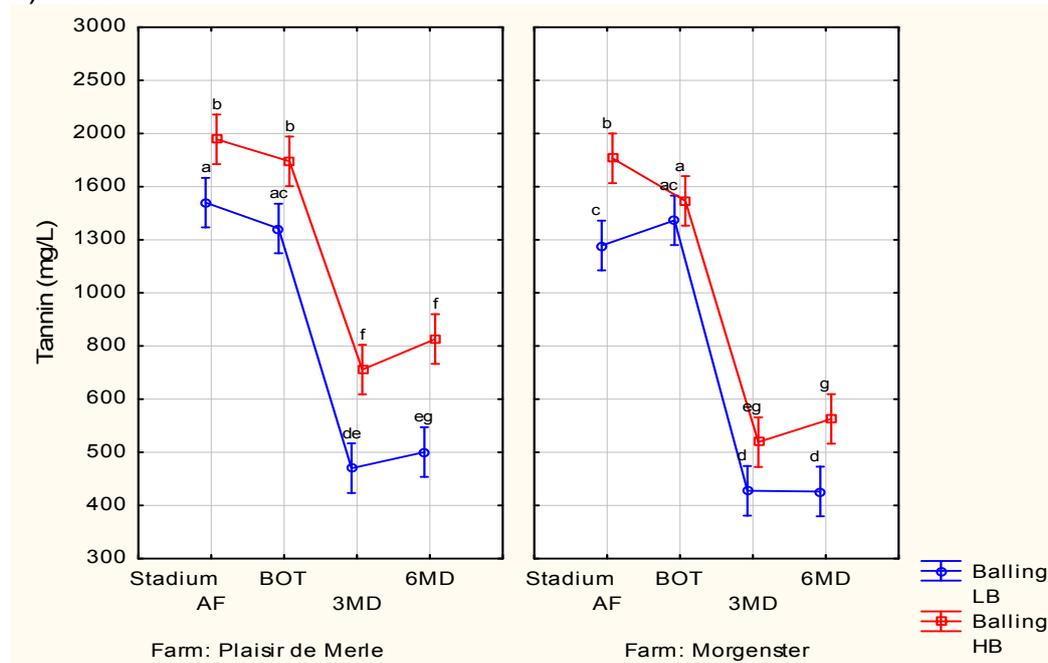
The effect of the different winemaking techniques on tannin concentration of the wines was also followed over time. In 2008, for both farms, there was a sharp drop in tannin concentration from BOT to 3MD. A more gradual decrease was observed in the 2009 harvest season (figure 3.6). The same trends (for both harvest seasons) were observed for the Shiraz wines (data not shown).

The most tannin is extracted after alcoholic fermentation (Cheynier *et al.*, 2006) and with a prolonged skin contact time after AF (Cheynier *et al.*, 2006). The majority of the tannins extracted at that time would be monomeric pigments consisting of catechin, epicatechin, epigallocatechin and epicatechin-gallate (Schofield *et al.*, 2001 & Sarneckis *et al.*, 2006). These four basic flavonoids are used in the formation of the different polymeric pigments. During the extraction time these monomers form dimers and trimers. As the wine matures, these dimers and trimers form longer chains of tannins and as the chains became larger it start to precipitate (Scollary, 2010). Tannins

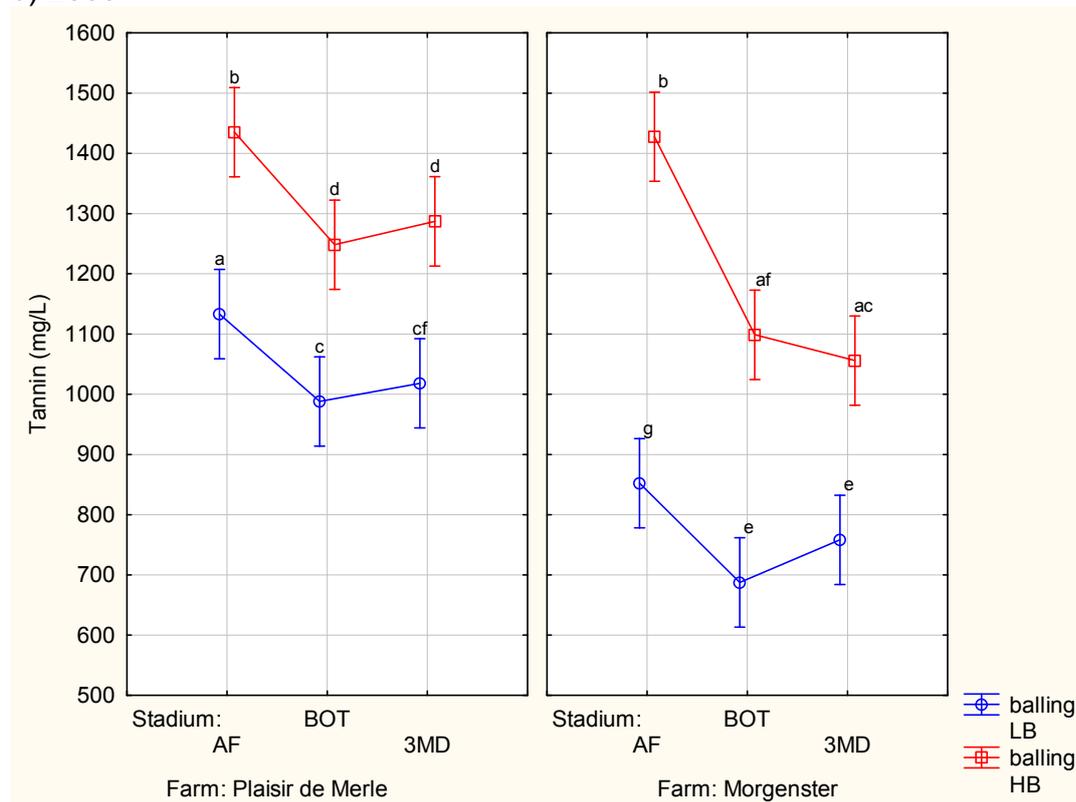
associate with anthocyanins to form polymeric pigments (Scollary, 2010). These polymeric pigments change from the blue/purple colour of young wines to the red/brown colour of matured wines (Scollary, 2010). The polymeric pigments are less sensitive to SO<sub>2</sub> bleaching and a shift in the pH balance (Scollary, 2010). This could explain the decline in tannin concentration from BOT to 3MD. After 3MD the polymerization of the tannins starts to stabilize, explaining the reason why there is no significant difference between 3MD and 6MD.

The BSA method also shows that the most tannin concentration was measured after AF and that there was a decline after BOT to 3 MD (data not shown). At both ripeness levels the tannin concentration stabilizes as the wine matures. The only differences between the ripeness levels are the concentration levels. The tannin concentration after AF was low and as the wine was bottled and matures, the tannin concentration stabilizes. This will occur due to polymerization that took place after bottling. This trend was consistent between the two cultivars (data not shown).

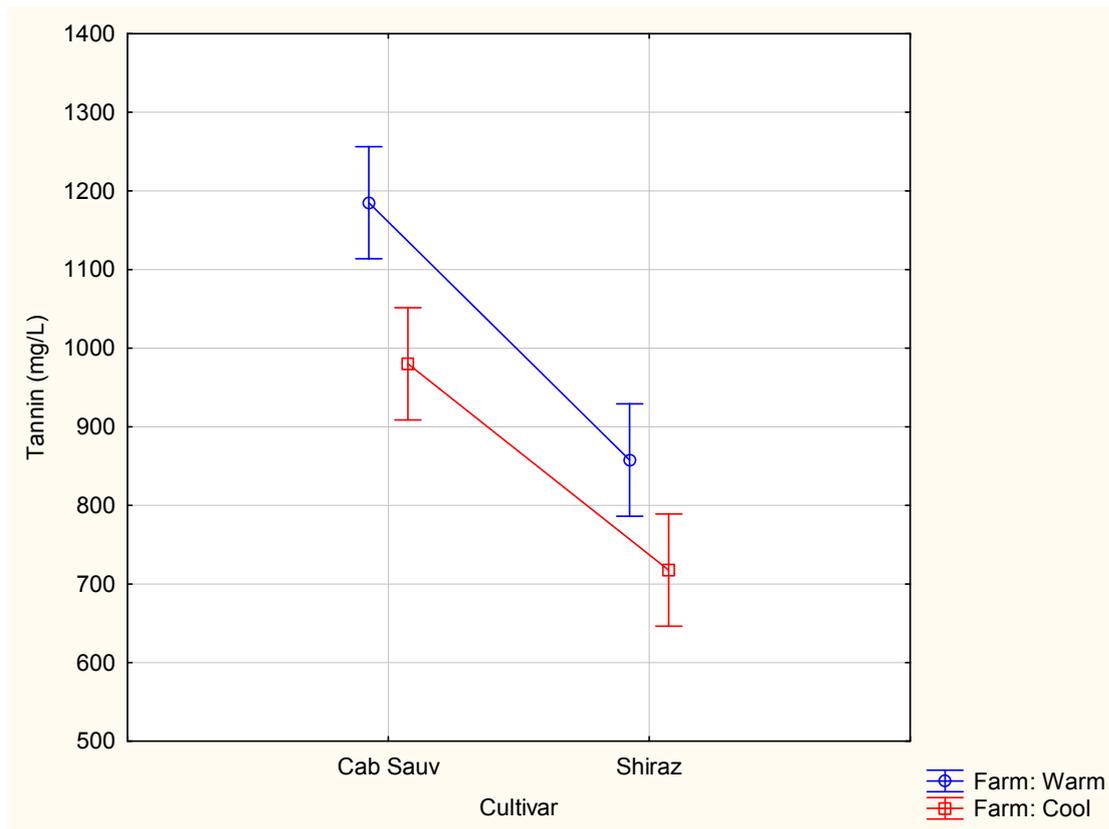
a) 2008



b) 2009



**Figure 3.6:** The average tannin concentration of all the treatments (MCP assay) of Cabernet Sauvignon of each farm of the 2008 harvest season (a). The treatments are: AF – alcoholic fermentation, BOT – bottling, 3MD – three months maturation and 6MD – six months maturation. In 2008 the Cabernet Sauvignon for LB – 22.7°B for Plaisir de Merle (harvested on 26 February) and 20.5°B for Morgenster (harvested on 4 March), HB – 27.1°B for Plaisir de Merle (harvested on 28 March) and 23.5°B for Morgenster (harvested on 4 April). In the 2009 harvest season the Cabernet Sauvignon for (b) LB – 20.9°B for Plaisir de Merle (harvested on 6 February) and 21.1°B for Morgenster (harvested on 12 March), HB – 23.8°B for Plaisir de Merle (harvested on 2 March) and 23.0°B for Morgenster (harvested on 14 April).



**Figure 3.7:** Total tannin concentration of all the treatments and stadiums of the two cultivars (Cabernet Sauvignon and Shiraz) on the two farms (Plaisir de Merle and Morgenster) of the 2009 harvest season.

Figure 3.7 show that Cabernet Sauvignon has overall a higher concentration of tannin than Shiraz. This also confirms previous results that the warmer farm has a higher tannin concentration than the cooler farm. This trend is the same for 2008 (data not shown) and 2009.

### 3.4.3.2 THE EFFECT OF DIFFERENT WINEMAKING TECHNIQUES ON TOTAL ANTHOCYANINS

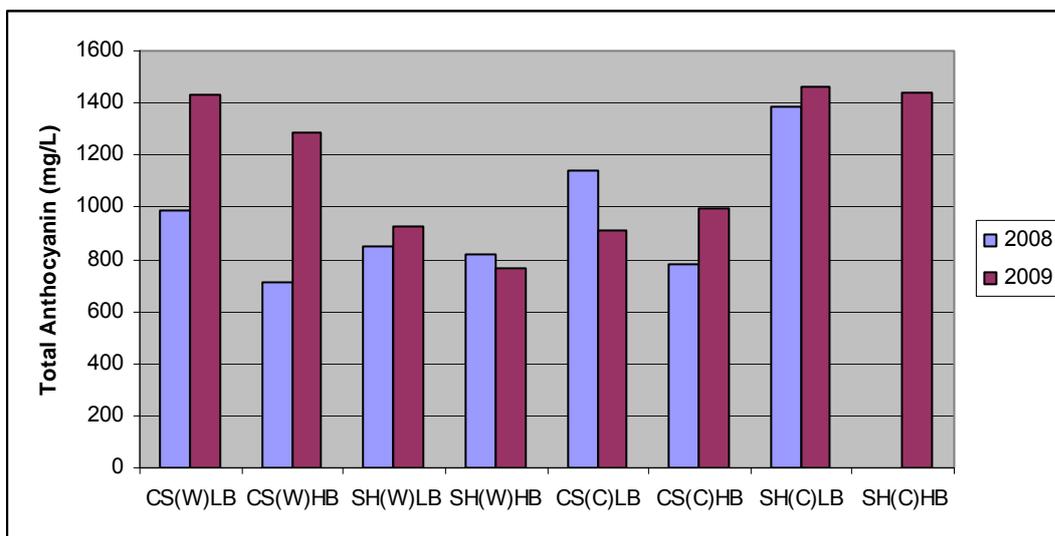
#### *Grapes*

Anthocyanins accumulate in the vacuoles of the epidermic cells of a grape berry (Gonzales-Neves *et al.*, 2008). These anthocyanins start to accumulate at veraison and can reach a peak from 40-50 days after veraison (Ryan and Revilla, 2003). Agro-ecological factors like cultivars, climate, canopy management and irrigation can be the reason for the different time in anthocyanin peaking (Gonzales-Neves *et al.*, 2008).

Figure 3.8 show that the highest anthocyanin levels was at the lower ripeness level and at the higher ripeness level the anthocyanin concentration decreased. This could mean

that the anthocyanins already reached their maximum and started to decline depicting phenolic ripeness (Ortega-Regules *et al.* 2006). The anthocyanin concentration of the Cabernet Sauvignon grapes from the cool farm actually increased in 2009. The cooler farm are subjected to a cool sea breeze from the Atlantic ocean that makes the growing and ripening of the grape berries longer than on the warmer farm. Therefore the grapes were still busy with anthocyanin accumulation and were not ready for the harvest.

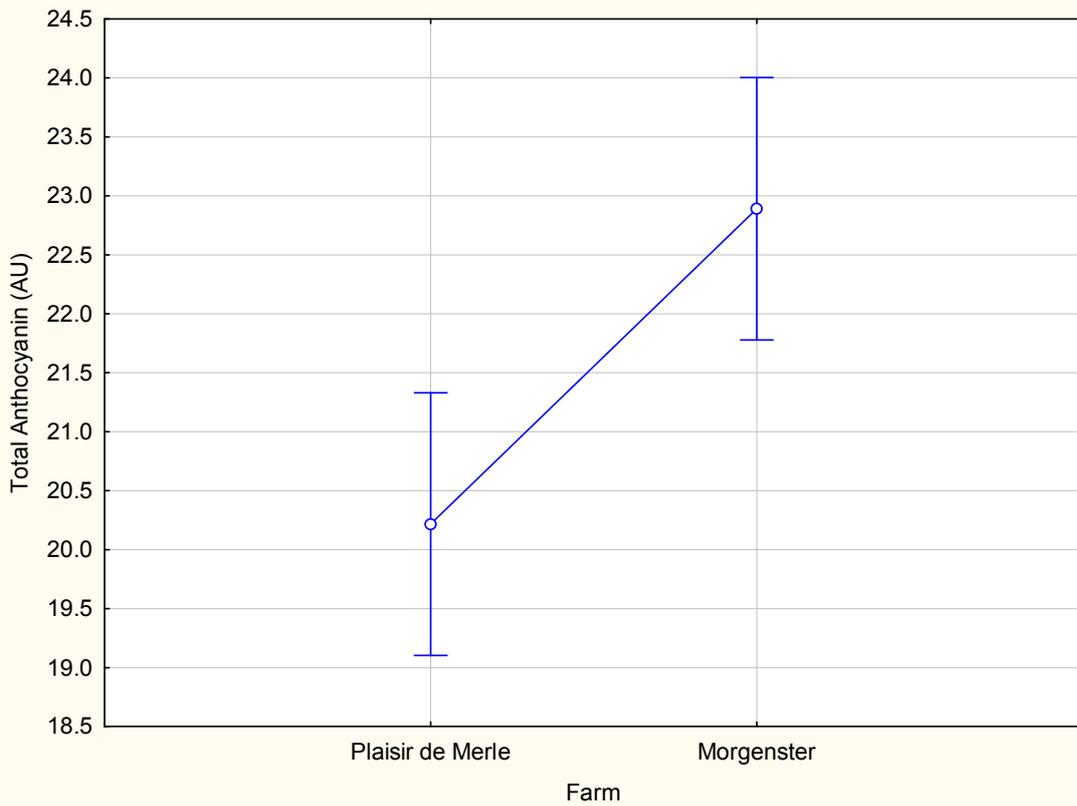
Anthocyanin accumulation is inhibited at warm temperatures above 26°C, but is expressed at low temperatures of 17°C-26°C, especially cool night temperatures of 15°C-20°C (Jackson and Lombard, 1993, Mori *et al.*, 2005 & Chorti *et al.*, 2010). In figure 3.9 one can see that the grapes from the cooler farm show a much higher anthocyanin concentration than the warmer farm.



**Figure 3.8:** Total anthocyanin (mg/L) of Cabernet Sauvignon (CS) and Shiraz (SH) that was harvested in 2008 and 2009 at two ripeness levels (LB and HB) on a warm (W) and cool (C) farm.

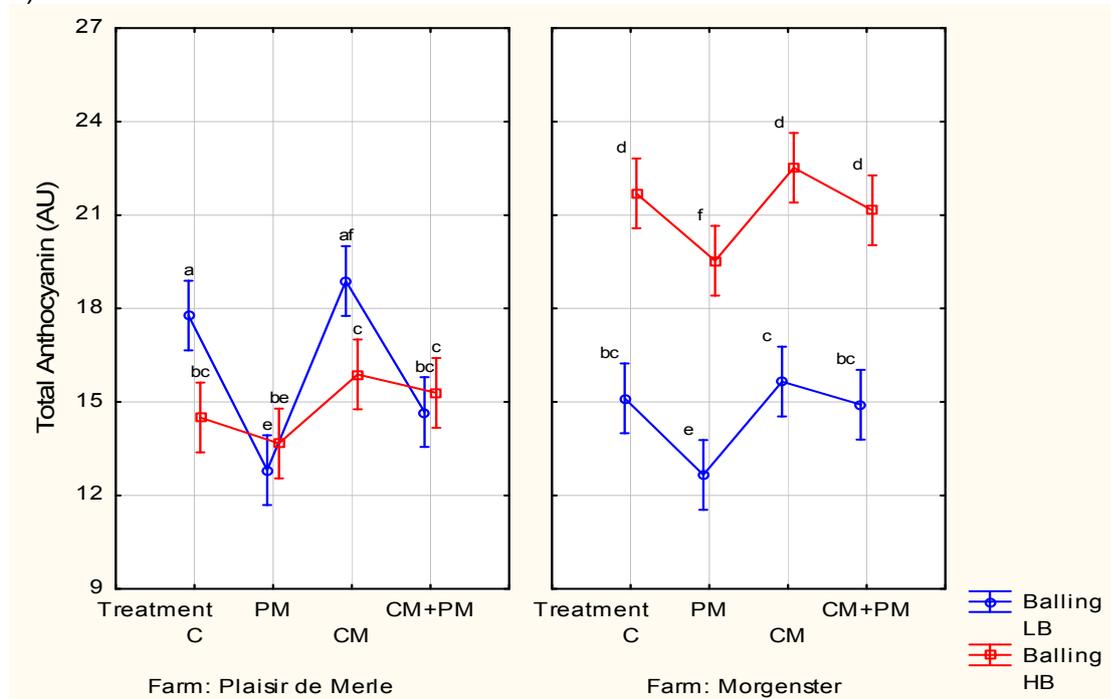
#### *Extraction treatments in winemaking*

Anthocyanin, in contrast to tannin, is water soluble and is extracted during cold maceration. Anthocyanin does not form bonds with cell wall structures and accumulate in the vacuoles of the epidermic cells (Gonzales-Neves *et al.*, 2008). Hence the red colour of grape skins.

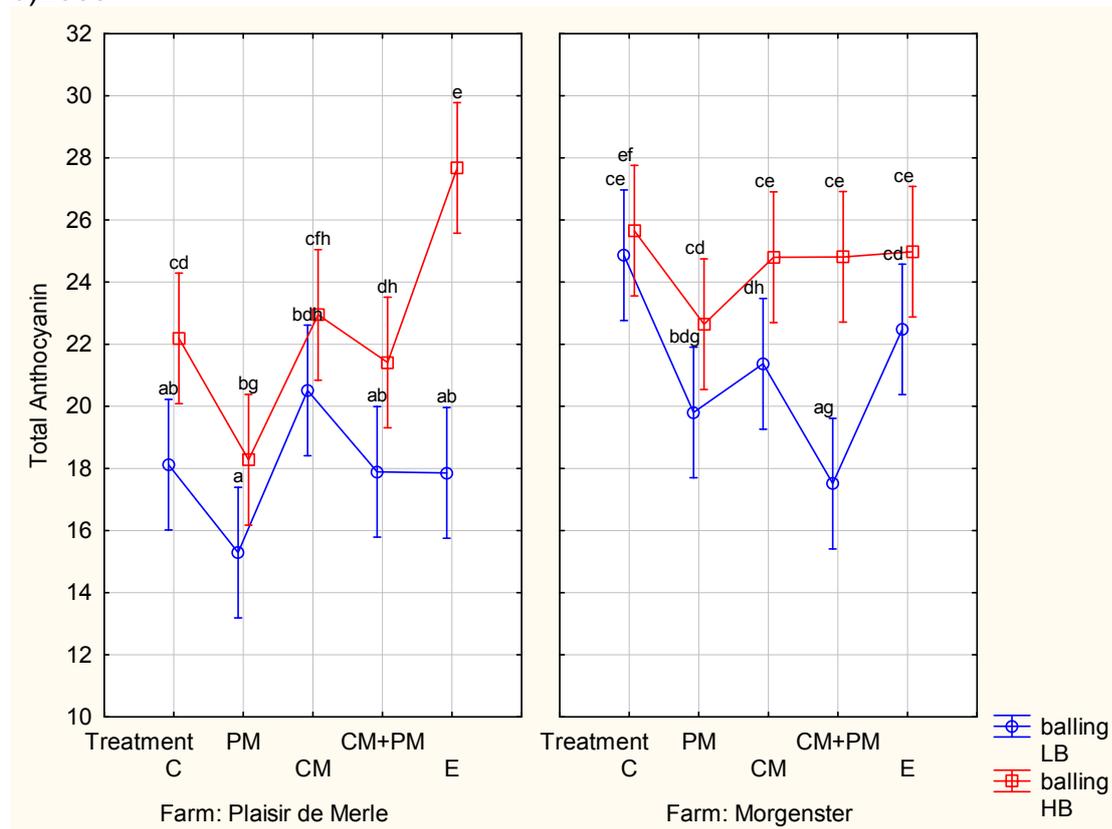


**Figure 3.9:** Total anthocyanin of all the treatments of the wines from the two farms.

a) 2008



b)2009

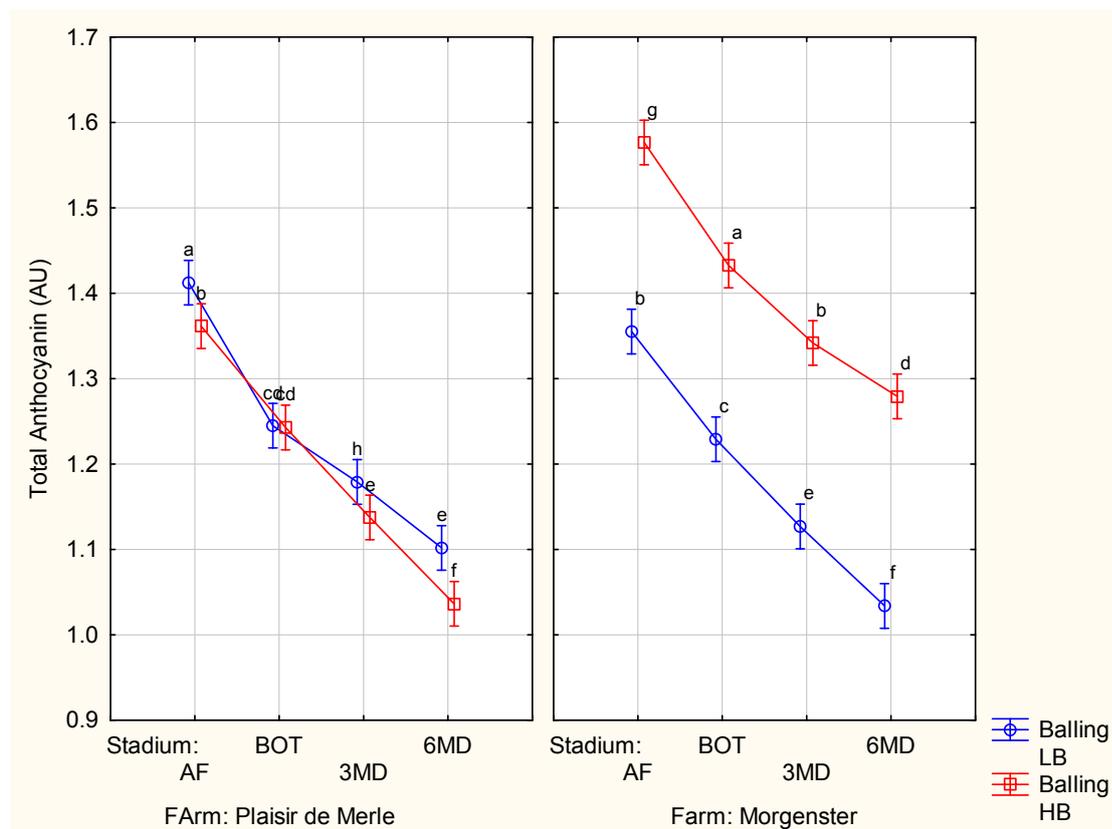


**Figure 3.10:** The total anthocyanin concentration of Cabernet Sauvignon wine of each farm of the 2008 harvest season. The treatments are: C – control wine, PM – post maceration, CM – cold maceration, CM+PM – combination of cold and post maceration. In 2008 the Cabernet Sauvignon for LB – 22.7°B for Plaisir de Merle (harvested on 26 February) and 20.5°B for Morgenster (harvested on 4 March), HB – 27.1°B for Plaisir de Merle (harvested on 28 March) and 23.5°B for Morgenster (harvested on 4 April). In the 2009 harvest season the Cabernet Sauvignon for (b) LB – 20.9°B for Plaisir de Merle (harvested on 6 February) and 21.1°B for Morgenster (harvested on 12 March), HB – 23.8°B for Plaisir de Merle (harvested on 2 March) and 23.0°B for Morgenster (harvested on 14 April).

At the 2008 harvest season [figure 3.10 (a)], one can see that at the warmer farm the PM treatment show a negative effect resulting in low concentration of total anthocyanin. The CM treatment didn't show an effect on total anthocyanin for the low ripeness level. This effect was evident on the cooler farm either at the low or higher ripeness levels. This indicates that cold maceration have no effect on total anthocyanin. At both farms and at both ripeness levels, the PM treatment had the lowest amount of anthocyanin concentration.

The 2009 [figure 3.10 (b)] wines from the warmer farm treated with the CM treatment had also no visible effect on total anthocyanin extraction at a low ripeness level and at a high ripeness level. The riper the grapes become, the quicker anthocyanin will be released, ending in an equal amount of anthocyanins, no matter the treatment (Glories, 1984). The E treatment showed no effect at a low ripeness level, but there was a remarkable increase in anthocyanin extraction at a higher ripeness level.

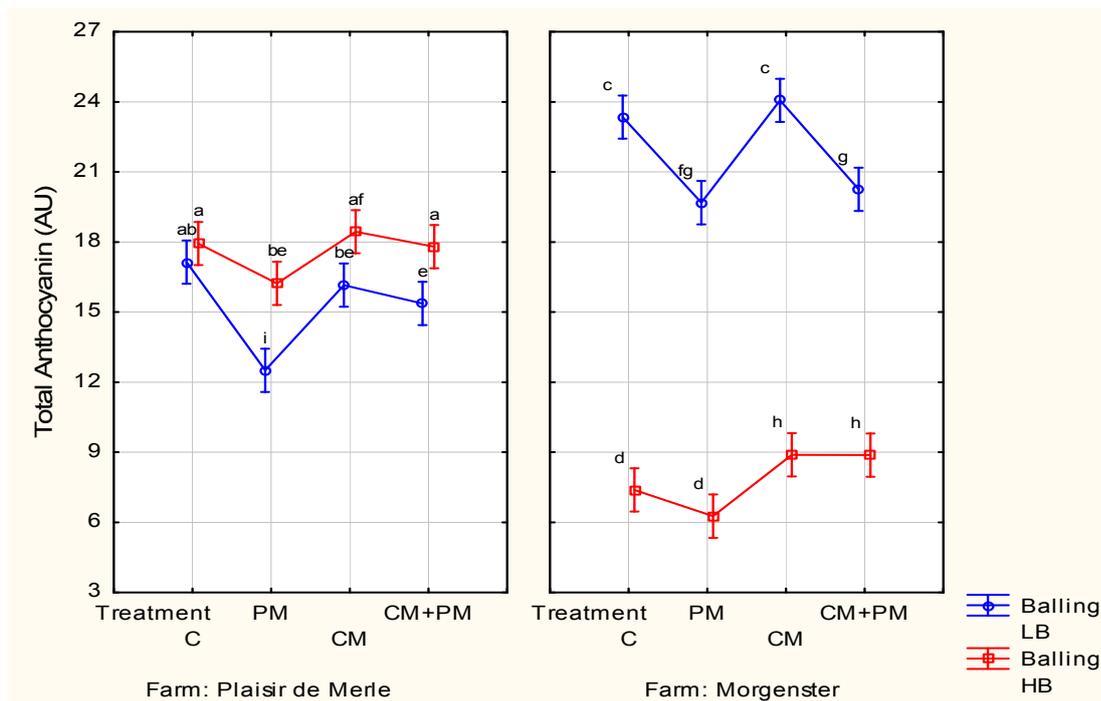
At the 2008 harvest season the cooler farm showed that at a low ripeness level, none of the treatments had any effect, except the PM treatment that showed a negative effect. Most of the anthocyanins were extracted by normal winemaking processes. There was a remarkable decline in anthocyanin concentration by the combined CM+PM treatment, probably due to reaction with tannin or other polymerization effects. At the higher ripeness level on the cooler farm, no treatment showed any effect. This is also probably due to a better release of anthocyanins as the grapes grew riper.



**Figure 3.11:** The average of total anthocyanin concentration of Cabernet Sauvignon wines of each farm of the 2008 harvest season. The stadiums are: AF – alcoholic fermentation, BOT – after bottling, 3MD and 6MD – three and six months. In 2008 the Cabernet Sauvignon for LB – 22.7°B for Plaisir de Merle (harvested on 26 February) and 20.5°B for Morgenster (harvested on 4 March), HB – 27.1°B for Plaisir de Merle (harvested on 28 March) and 23.5°B for Morgenster (harvested on 4 April).

In 2008, on the warmer farm, there was a decline of anthocyanin (figure 3.11) concentration as the wine matured. This can be expected as anthocyanins form polymeric pigments with tannins and also shift in the colour spectrum from red to yellow/brown as measured by a spectrophotometer. On the warmer farm the anthocyanin concentration was the same for both the low and high ripeness levels. For the cooler farm, the decline in anthocyanin concentration was also observed, but there was a difference in concentration between the ripeness levels.

At the 2009 harvest season the same trend can be seen (data not shown) for both farms. On both farms the anthocyanin concentration is lower for low ripeness levels and higher for higher ripeness levels. The steepest decline is from AF to BOT as it is when the wine was filtered and bottled and the ingress of oxygen will show a decline in anthocyanin concentration as acetaldehyde is formed (Scollary, 2010). This is also the time when polymerization takes place. From BOT to 3MD the decline is more gradually as the anthocyanins started to become more stable.



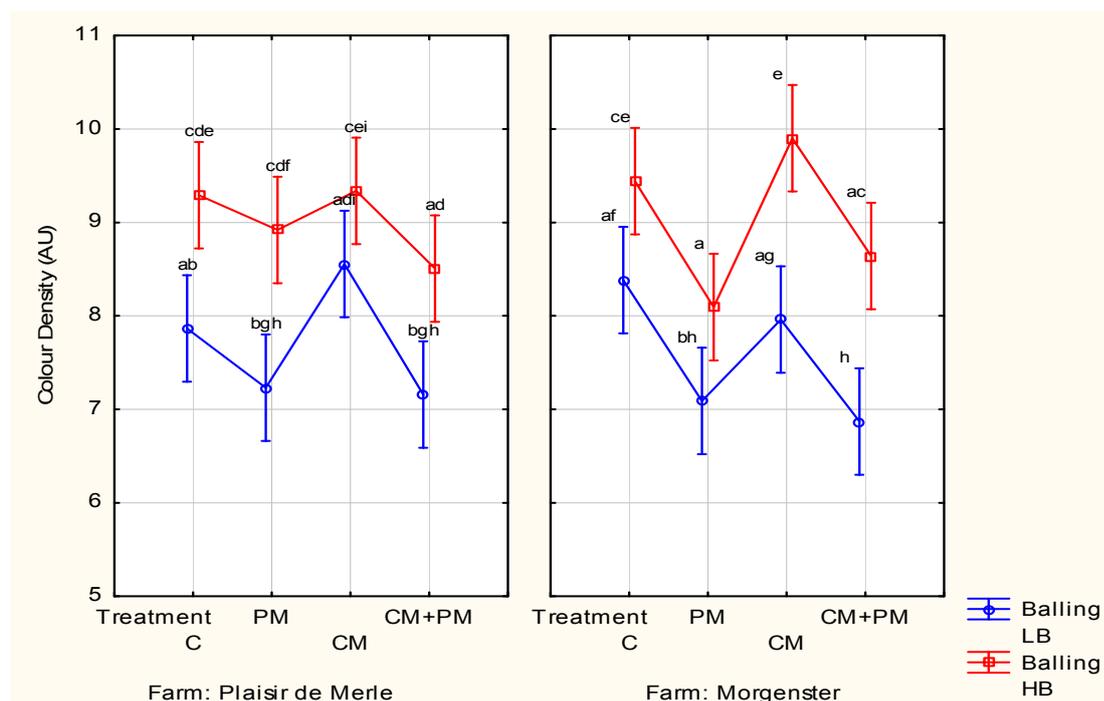
**Figure 3.12:** The total anthocyanin concentration of Shiraz wines of each farm of the 2008 harvest season. The treatments are: C – control wine, PM – post maceration, CM – cold maceration, CM+PM – combination of cold and post maceration. In 2008 the Shiraz for LB – 24.0°B for Plaisir de Merle (harvest on 4 March) and 20.5°B for Morgenster (harvest on 23 February), HB – 27.0°B for Plaisir de Merle (harvest on 28 March) and 24.4°B for Morgenster (harvest on 5 March).

The Shiraz was an interesting case in 2008. For the most part, it followed a similar trend to Cabernet Sauvignon. Figure 3.12 show that there was no difference in the extraction of anthocyanins of the CM and CM+PM treatments at a higher ripeness level from the warmer area. This was not the case for the cooler area where the CM and CM+PM treatments show an increase in anthocyanin extraction. Again the PM treatment showed the lowest anthocyanin concentration, also for both farms. On the warmer farm the concentration difference for the ripeness levels are quite small, with the low ripeness level showing the lowest concentration. The difference in anthocyanin concentration for the cooler farm is very large. This shows that the concentration for the ripe level is much lower than for the low ripeness level. This could be partially be explained by the

respective healthiness of the grapes. The Shiraz grapes on the warmer farm were healthy and looked after. On the other hand, the Shiraz grapes on the cooler farm were overcropped and Botrytis was evident at a lower ripeness level and became worse as the grapes ripen. When the grapes were picked at the high ripeness levels, the grapes were rotten to a point where it was impossible to produce good wine. This could be the reason for the low anthocyanin concentration for the cooler farm at the high ripeness level. In 2009 the same trend of the effect of the different treatments on total anthocyanins can be seen (data not shown) as for the 2008 harvest season. The same trend on the anthocyanin concentration can be seen (data not shown) for Cabernet Sauvignon.

### 3.4.3.3 THE EFFECT OF DIFFERENT WINEMAKING TECHNIQUES ON COLOUR DENSITY

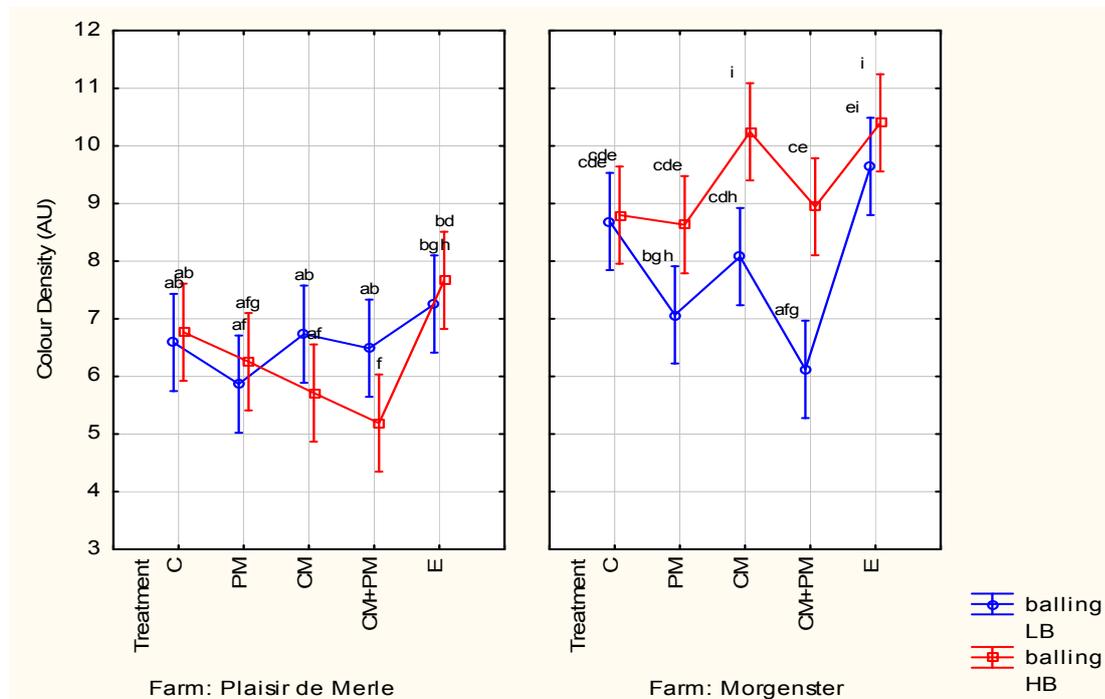
#### Extraction treatments in winemaking



**Figure 3.13:** The colour density of Cabernet Sauvignon of each farm of the 2008 harvest season. The treatments are: C – control wine, PM – post maceration, CM – cold maceration, CM+PM – combination of cold and post maceration. In 2008 the Cabernet Sauvignon for LB – 22.7°B for Plaisir de Merle (harvested on 26 February) and 20.5°B for Morgenster (harvested on 4 March), HB – 27.1°B for Plaisir de Merle (harvested on 28 March) and 23.5°B for Morgenster (harvest on 4 April).

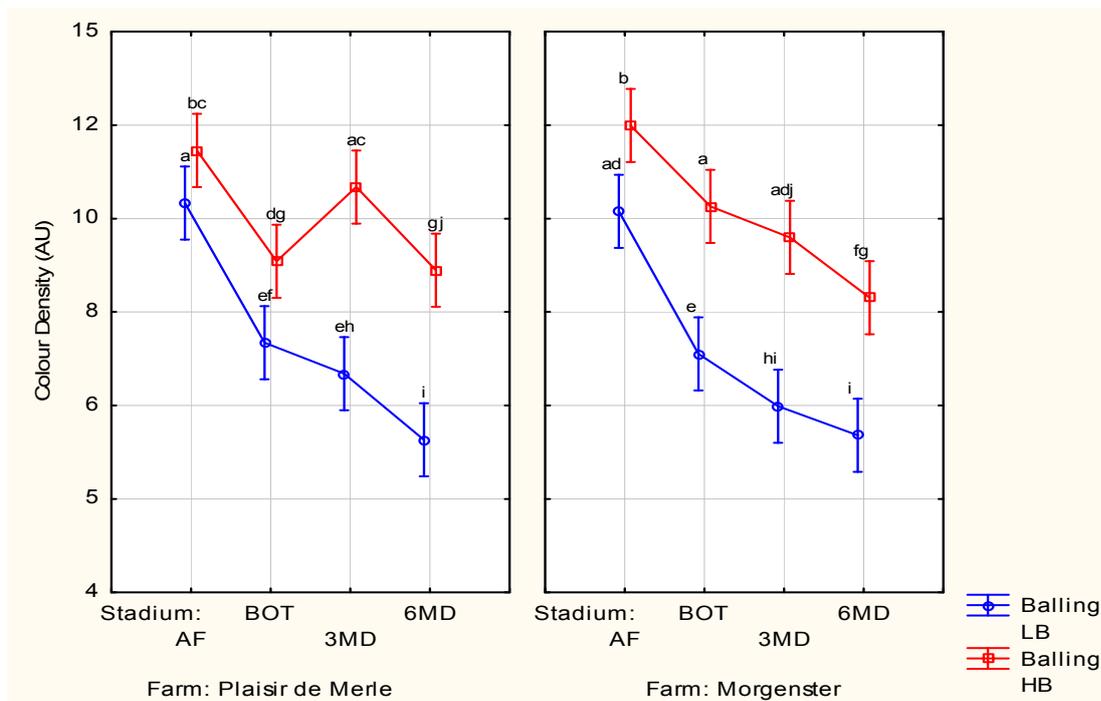
In figure 3.13 the PM treatment as well as the combined CM+PM treatment show a negative effect in colour density, while the CM treatment show a slight increase in colour density. This is evident for both the farms. The effect was more significant for the

cooler farm. The same trend could be seen with the Shiraz grapes of the two farms (data not shown). During the 2009 harvest season, none of the treatments showed any effect on colour density from the higher ripeness level (data not shown). The tendency for CM treatment to be higher than the PM and CM+PM treatments can be seen for both the total anthocyanins and the colour density.



**Figure 3.14:** The colour density of Shiraz of each farm of the 2009 harvest season. The treatments are: C – control wine, PM – post maceration, CM – cold maceration, CM+PM – combination of cold and post maceration. In the 2009 harvest season the Shiraz for (b) LB – 23.4°B for Plaisir de Merle (harvested on 18 February) and 21.8°B for Morgenster (harvested on 24 March), HB – 24.6°B for Plaisir de Merle (harvested on 2 March) and 24.4°B for Morgenster (harvested on 14 April).

During the 2009 harvest, the colour density showed different trends than those observed during the 2008 harvest season. For both cultivars (Cabernet Sauvignon and Shiraz) of the warmer farm, the treatments show no effect on colour density, with only the E treatment (figure 3.14) that shows a significant increase in colour density. On the cooler farm the treatments of CM and E show the same increase in colour density for both cultivars. The CM treatment shows again the same tendency to be slightly higher than the PM treatment for both the total anthocyanins and colour density. The E treatment also show the highest extraction, while in the cooler area the CM+PM treatment show a negative effect in both the total anthocyanins and colour density.



**Figure 3.15:** The colour density of all the treatments of Cabernet Sauvignon of each farm of the 2008 harvest season. The treatments are: The stadiums are: AF – alcoholic fermentation, BOT – after bottling, 3MD and 6MD – three and six months. In 2008 the Cabernet Sauvignon for LB – 22.7°B for Plaisir de Merle (harvested on 26 February) and 20.5°B for Morgenster (harvested on 4 March), HB – 27.1°B for Plaisir de Merle (harvested on 28 March) and 23.5°B for Morgenster (harvest on 4 April).

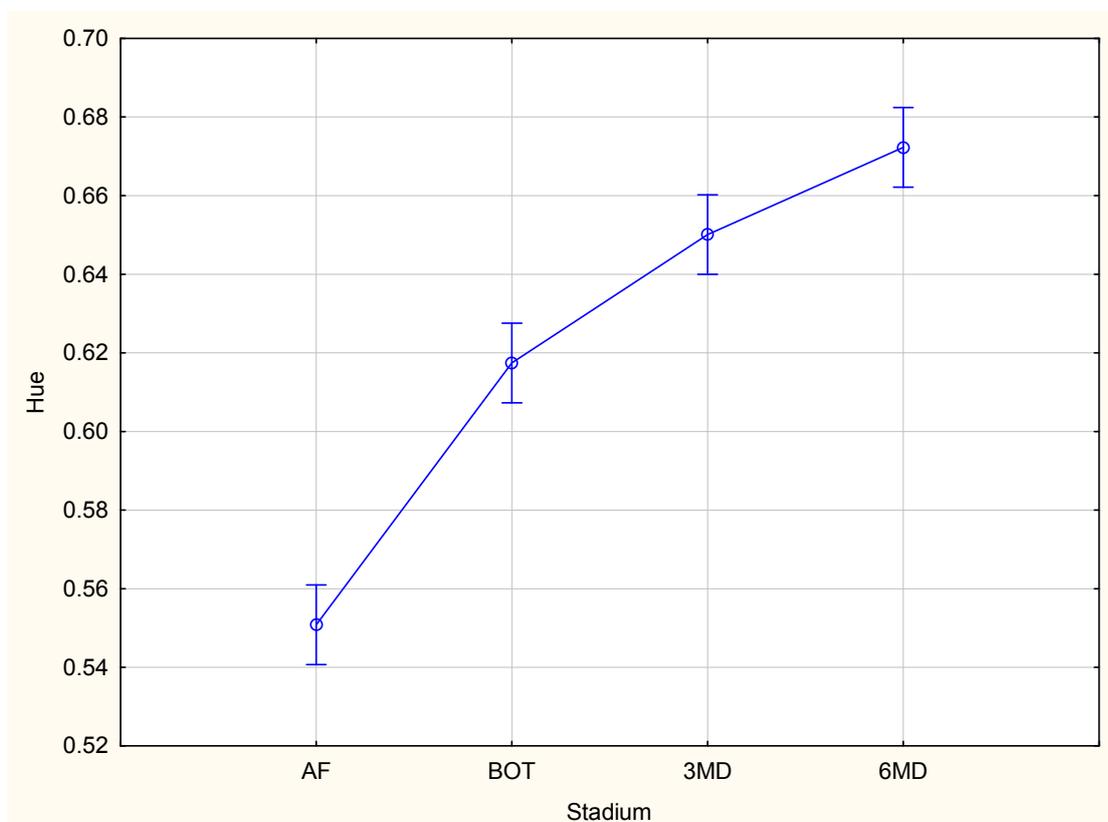
On both farms, for both the cultivars and for both the 2008 and 2009 harvest seasons, the colour density is high just after AF. As can be seen in figure 3.15 there is a sharp decrease in colour density to BOT after which it decreases even further. These decreases can be due to the formation of polymeric pigments at the bottling stage (Somers, 1971). The concentration of anthocyanin kept on decreasing probably indicating polymerization with tannins (Somers, 1971). This tendency of loss in colour density as the wine matures can also be seen with total anthocyanins. The same results were obtained for Shiraz (data not shown).

According to Ribéreau-Gayon *et al.* (2000) colour density has a tendency to increase when the anthocyanins come in contact with air or oxygen. This may explain the increase in colour density for the higher ripeness level at three months maturation on the warmer farm (Ribéreau-Gayon *et al.*, 2000).

### 3.4.3.4 THE EFFECT OF DIFFERENT WINEMAKING TECHNIQUES ON COLOUR INTENSITY/HUE

#### *Extraction treatments in winemaking*

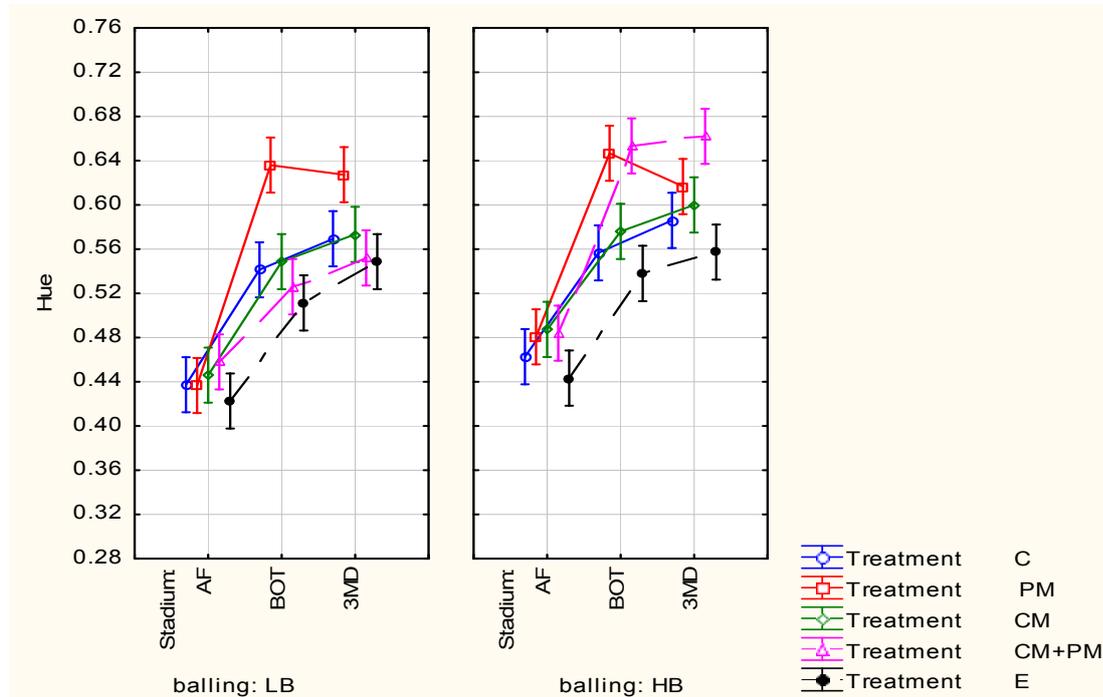
Anthocyanins absorb at 420 nm and 520 nm in the visible spectrum. These wavelengths correlate to the yellow/brown and red spectrum respectively (Margalit, 1997). Colour intensity (or hue) is the ratio between 420 nm and 520 nm. Young red wine have values in the order of 0.5-0.7, which increases throughout the maturation time to reach values of 1.2-1.3 (Ribéreau-Gayon *et al.*, 2000). Hue can therefore be used to access if a wine went through a maturation time (figure 3.16) or if the wine is oxidized.



**Figure 3.16:** A graph depicting the increasing hue values as wine matures. The values for hue are an average for all the stadiums. The trend was the same for the cultivars Cabernet Sauvignon and Shiraz and for both the farms (Plaisir de Merle and Morgenster).

The average hue for the 2008 harvest season (data not shown) was 0.55 for the cultivars Cabernet Sauvignon (warmer and cooler farm) and Shiraz (warmer farm). This was also the case for both ripeness levels. This means that the ripeness level had no effect on the evolution of the hue of the wine. What did have an effect on hue was botrytis infection of the Shiraz on the cooler farm. The hue started at 0.65 for the low ripeness level and jumped to 0.80 for the higher ripeness level. This means that laccase had an oxidizing effect on the colour of the wine, increasing the yellow/brown spectrum

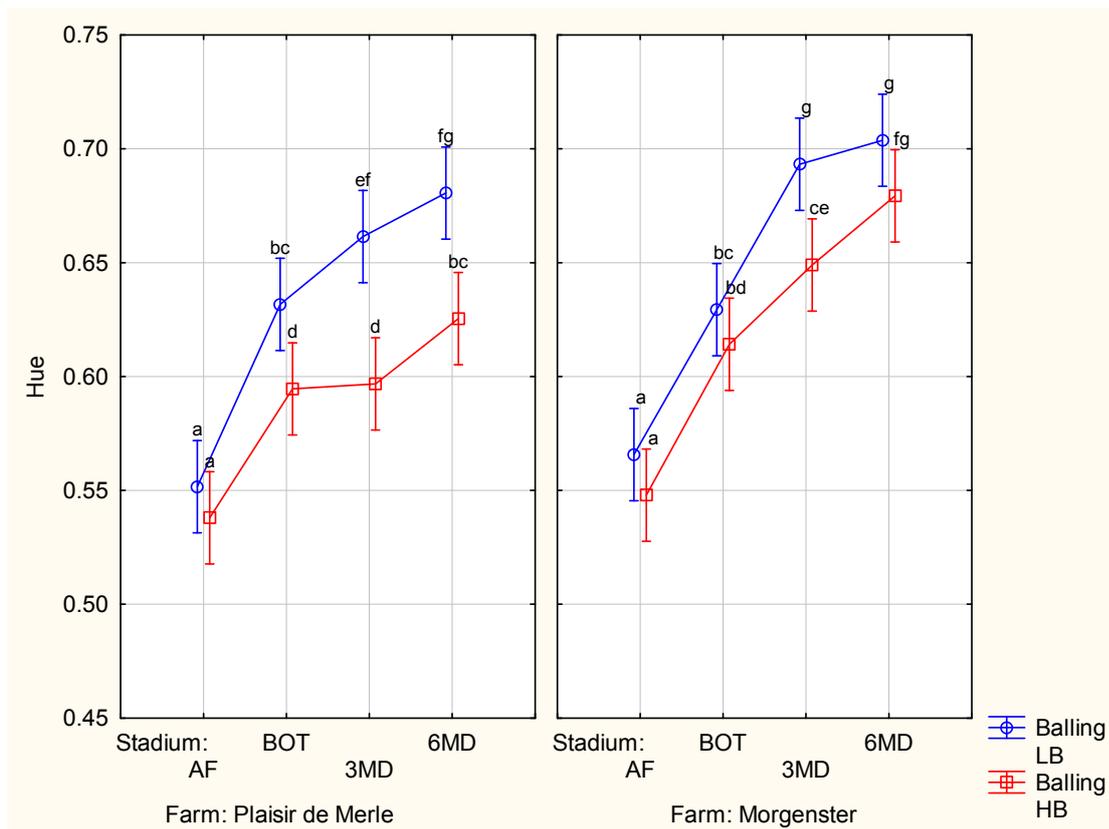
and decreasing the red. Laccase transforms the phenolic compounds (caffeic and p-coumaric acids which are esterified with tartaric acid) to quinones. When these quinones polymerize, they form brown pigments (Ribéreau-Gayon *et al.*, 2000).



**Figure 3.17:** The hue of the treatments over the different stadiums of the different farms. The cultivar is Shiraz from the 2009 harvest season.

The evolution of hue follows the same trends in both the 2008 and 2009 harvest season (figure 3.17 for the 2009 harvest season). Figure 3.17 show that the E treatment shows the best results for hue and that the hue will be more stable than for instance wine that was made with the PM or CM+PM treatments.

At bottling (BOT) the hue increased significantly. The hue for the 2008 harvest was slightly higher (0.6-0.65) than the hue for the 2009 harvest (0.48-0.56). This increase in hue is expected as there is a notable increase in oxygen uptake during the bottling stage. The increase in hue after bottling was more subtle ending in a hue for Cabernet Sauvignon and Shiraz of 0.63-0.7 (2008) and 0.5-0.6 (2009) for the warmer farm, while it was 0.7-0.85 (2008) and 0.48-0.55 (2009) for the cooler farm. In 2008 the lower ripeness level has on average lower values for hue than the higher ripeness levels. In 2009 the opposite was true and here the higher ripeness levels have lower hue values than the lower ripeness levels.



**Figure 3.18:** The average hue for Cabernet Sauvignon of the 2008 harvest for the two farms. The treatments are: The stadiums are: AF – alcoholic fermentation, BOT – after bottling, 3MD and 6MD – three and six months. In 2008 the Cabernet Sauvignon for LB – 22.7°B for Plaisir de Merle (harvest on 26 February) and 20.5°B for Morgenster (harvest on 4 March), HB – 27.1°B for Plaisir de Merle (harvest on 28 March) and 23.5°B for Morgenster (harvest on 4 April).

Hue increases with maturation. The PM treatment and the combined CM+PM treatment show increases in hue values for both farms and both cultivars (data not shown). This can be expected as the post maceration part involve a further skin contact time after fermentation was completed. During fermentation CO<sub>2</sub> are given off as a by-product of fermentation. This CO<sub>2</sub> blanket on top of fermenting skins protects the wine against oxidation. After fermentation, and especially in small scale winemaking, this protective CO<sub>2</sub> blanket quickly disappears and can be replace by O<sub>2</sub>, which in time will start to oxidize the wine resulting in a higher yellow/brown to red ratio, increasing the hue.

Again in the 2008 harvest season the higher ripeness level show on average a lower hue value than for the lower ripeness level. In the 2009 harvest season it was the opposite. The E treatment that was used in the 2009 harvest season, show a decrease in hue values. This could only mean that the pectolytic enzymes have a more stabilizing effect on colour and hue depicting in low hue values (data not shown). The E treatment has a more significant impact on hue values on a higher ripeness level on the warmer farm than on the cooler farm. There was no significant difference between the CM and

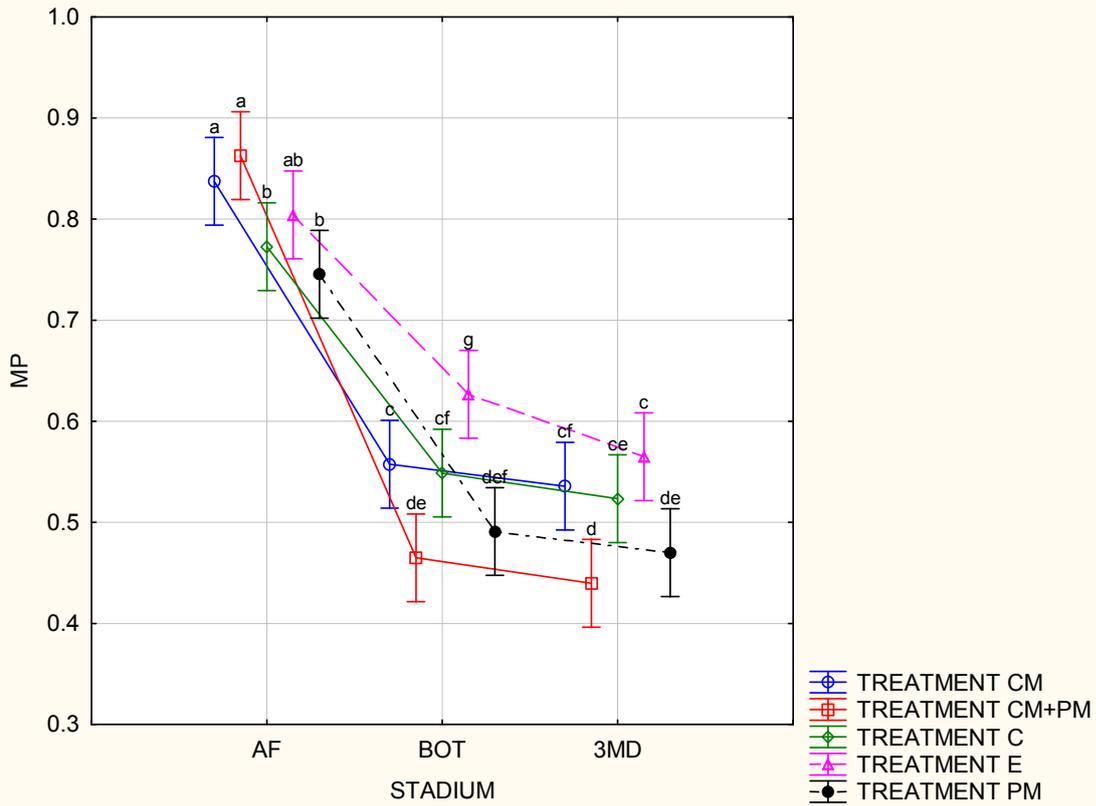
E treatment on the cooler farm. This means that because of a higher colour density of red wine from the cooler farm, enough of the red spectrum is extracted during the CM treatment as well as with the E treatment. The data show that on cooler farms it is not really necessarily to use an E treatment to influence the hue, but on warmer farms it would be beneficial.

#### **3.4.3.5 THE EFFECT OF DIFFERENT WINE MAKING TECHNIQUES ON MP, SPP AND LPP**

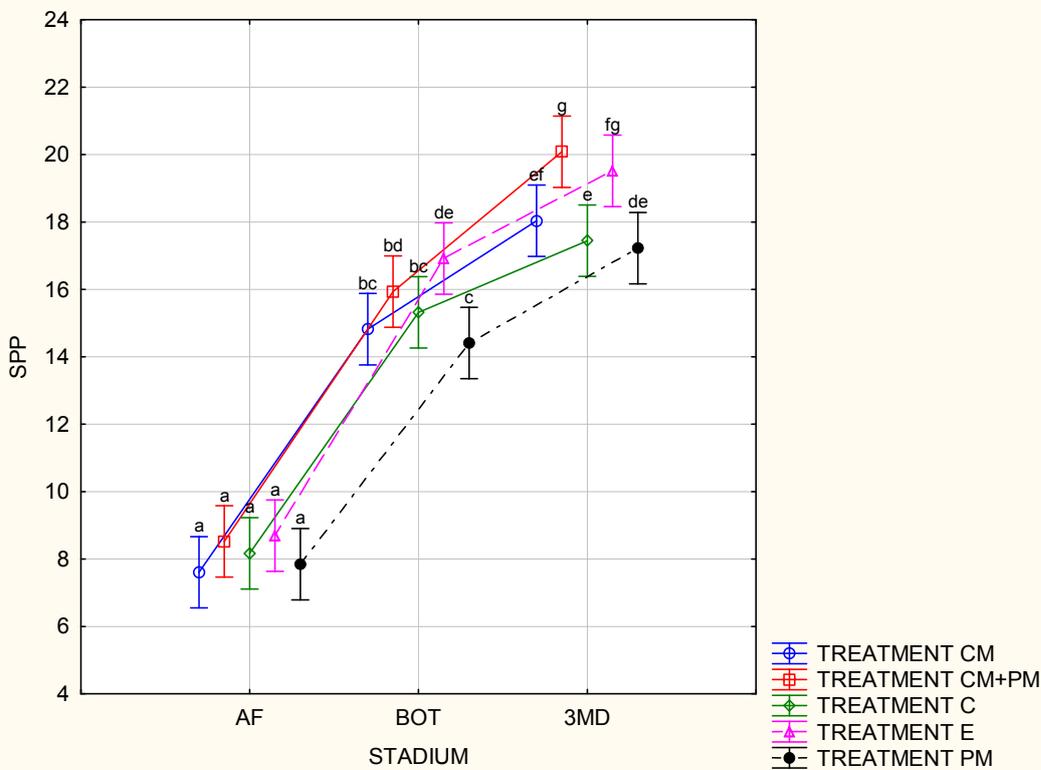
In 2003 Harbertson *et al.* combined bisulphite bleaching with the protein precipitation (BSA) assay. They did this to distinguish monomeric anthocyanins (MP) from polymeric pigments (PP). They found that there were two classes of polymeric pigments: small polymeric pigments (SPP) that do not precipitate with protein and large polymeric pigments (LPP) that do.

In figure 3.19 there are a sharp decrease in MP from AF to BOT. This would be that there are more monomeric anthocyanins during fermentation and after AF a polymerization took place producing polymeric pigments. The decrease from BOT to 3MD is less sharp as the anthocyanins start to stabilize. After the 3MD the treatment with the highest MP are the E treatment and the lowest the combined CM+PM treatment. This again shows the effect of enzymes in releasing anthocyanins from the cells (Gonzales-Neves *et al.*, 2008). During the combined CM+PM treatment, polymerization started at an early stage resulting in less MP. This can be seen for both cultivars and on both the farms (data not shown).

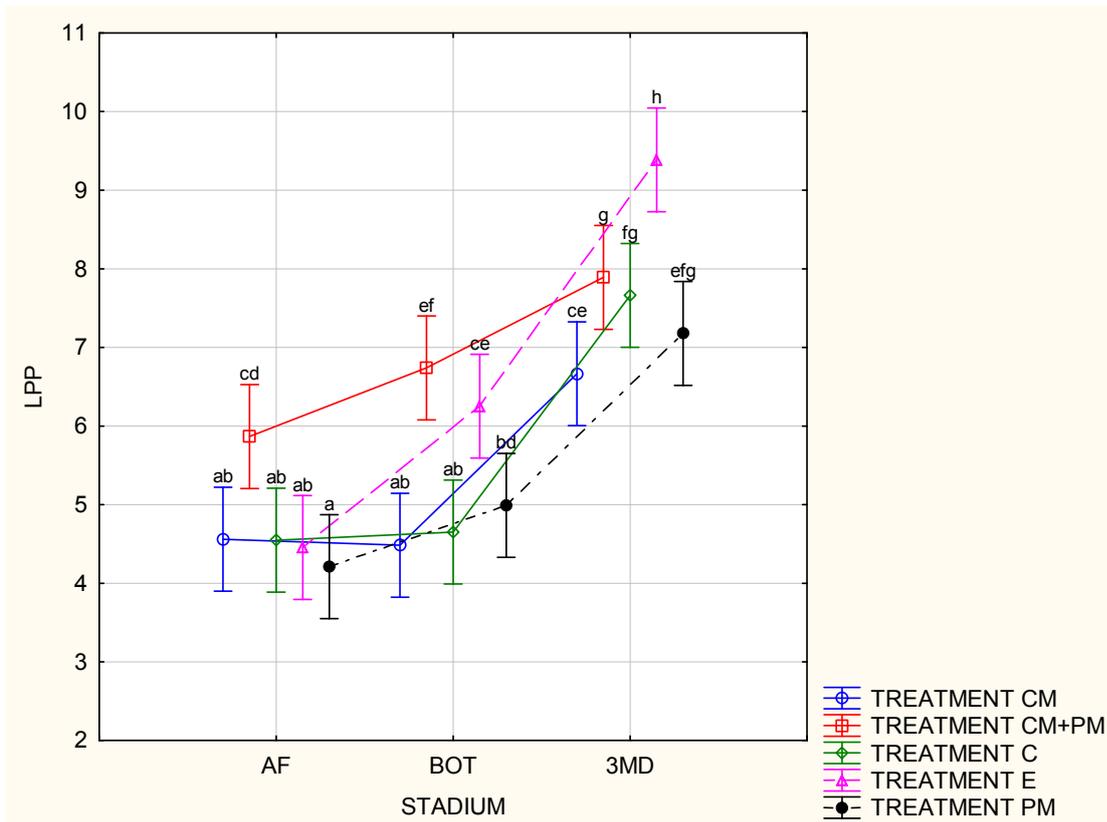
As the MP decreases with BOT to maturation (figure 3.19), SPP and LPP increases from AF to BOT to 3MD maturation. This was expected; as MP polymerizes (decrease) more PP (increase) will form. Therefore anthocyanins decrease gradually while SPP and LPP are formed and the anthocyanins are transformed in stable polymeric pigments (Villamor *et al.*, 2009). Harbertson *et al.* (2003) found that most of the SPP are formed after AF (figure 3.21).



**Figure 3.19:** The monomeric anthocyanins (MP) of Shiraz from the different stadiums to the different treatments of the 2009 harvest season.

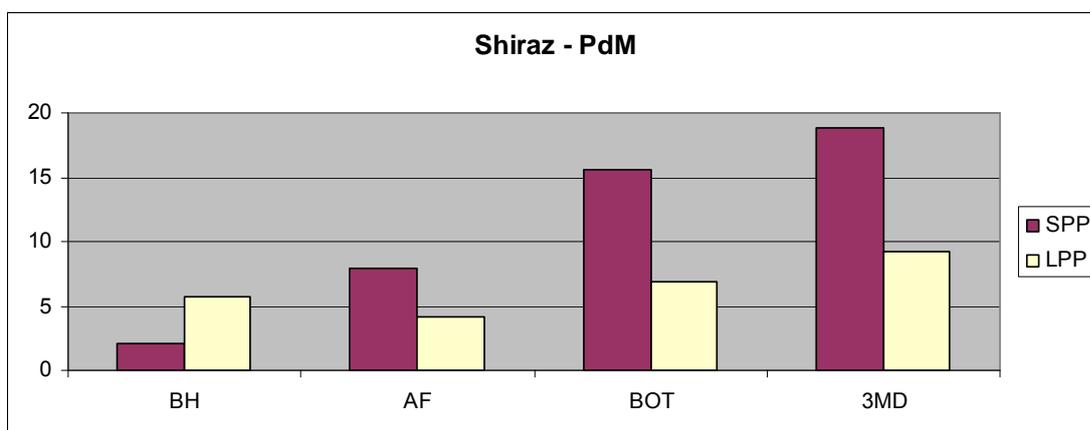


**Figure 3.20:** The small polymeric pigments (SPP) of Cabernet Sauvignon from the different stadiums from both farms and cultivars of the 2009 harvest season.

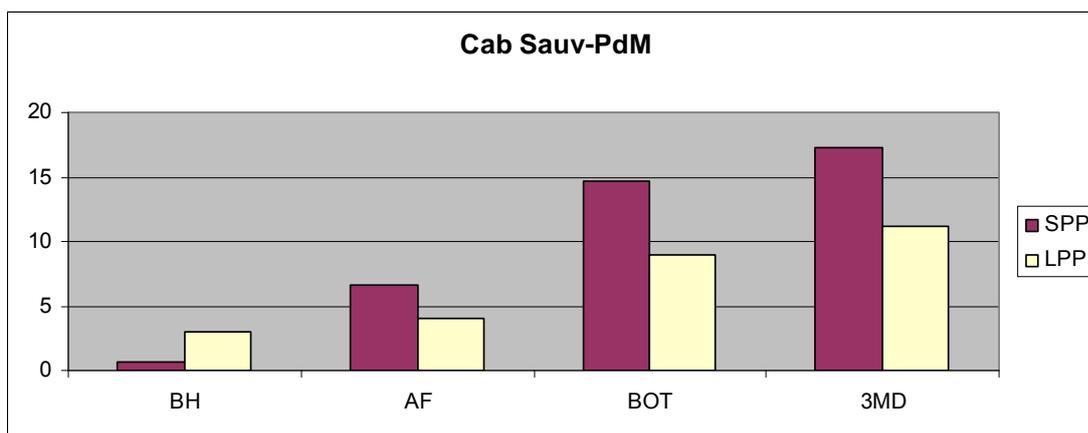


**Figure 3.21:** The large polymeric pigments (LPP) of Cabernet Sauvignon from the different stadiums to the different treatments of the 2009 harvest season.

According to Villamor *et al.* (2009) LPP are more favourably formed in bottled wines than SPP. This could explain the sharper increase in LPP from BOT to 3MD (figure 3.21) than the more evenly increase of SPP from BOT to 3MD (figure 3.20). Mansfield and Zoecklein (2003) speculate that an increase in LPP indicates changes in phenolic structure similar found in aged wines. This may be proven by further studies with HPLC.



**Figure 3.22:** The average of small polymeric pigments (SPP) and large polymeric pigments (LPP) of Shiraz from Plaisir de Merle measured over the different stadiums. BH – before harvest, AF – alcoholic fermentation, BOT - bottling and 3MD – 3 months maturation.



**Figure 3.23:** The average of small polymeric pigments (SPP) and large polymeric pigments (LPP) of Cabernet Sauvignon from Plaisir de Merle measured over the different stadiums. BH – before harvest, AF – alcoholic fermentation, BOT - bottling and 3MD – 3 months maturation.

The colour of red wine consists of anthocyanins and their acylated forms. These anthocyanins are very unstable and form copigments with other anthocyanins or procyanidins just after fermentation (Boulton, 2001). These monomeric anthocyanidins are very sensitive for sulphite bleaching (Harbertson *et al.*, 2003). So the expectation will be that at AF the MP concentration will be high and that the concentration will decrease as the wine matures (figure 3.19) and that these MP will form PP. According to Harbertson *et al.* (2003) there is a high concentration of MP in the grapes and the concentration of MP decreases after AF. In all the samples that Harbertson *et al.* (2003) analyzed the concentration of MP are always higher in grapes than for the corresponding wines. In our studies we found that there were no big differences in MP concentration of the grapes and the corresponding wines (data not shown).

The SPP are dimers, trimers and oligomers which are small molecular weight compounds that do not precipitate with a protein. Polymeric pigments can be formed by self association (Boulton, 2001) bonding, tannin-anthocyanin (Remy *et al.*, 2000) bonding, and acetaldehyde cross-linking (Saucier *et al.*, 1997). These SPP are resistant to SO<sub>2</sub> bleaching which may contain compounds like vitisins (Harbertson *et al.*, 2003 and Versari *et al.*, 2007). Harbertson *et al.* (2003) found that the concentration for SPP in the fruit is slightly higher than for the corresponding wine. In our case the SPP concentration in grapes are much lower than for the corresponding wines (figures 3.22 and 3.23). These concentrations keep increasing up to the 3MD maturation. An explanation could be that during the maturation process more and more dimers etc. are formed. The same was observed for the both cultivars at the two farms. A better understanding of this phenomenon can be obtained by HPLC analysis.

The LPP concentration of the fruit is much lower than for the corresponding wines (figures 3.22 and 3.23). This findings was the same what Harbertson *et al.* (2003) found. These LPP increase as the wine matures. Harbertson *et al.* (2003) confirmed that LPP are formed during fermentation and that LPP concentration will increase with maturation (Harbertson *et al.*, 2006). This are expected as PP are formed by the polymerization of monomers.

### 3.5 CONCLUSION

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In this study it was found that there were questions to be answered about the tannin extraction and the extractability index. But there is a lot of potential in the use of the Glories method as a parameter to determine harvest time. More studies are needed to investigate the different extractabilities and their correlation to tannin extraction of the different cultivars.

The MCP method for tannin analysis is a much quicker and easier method to use than the BSA method if you are interested in the tannin concentration of the wine. To investigate the MP, SPP and LPP of the wine, the BSA method is the only method to use. As Sacchi *et al.* (2005) stated that there were a lot of studies done on the effect of winemaking techniques but these studies don't include the effect on polymeric pigments.

The different winemaking treatments had different results on the extraction of tannins and anthocyanins of the two cultivars and at two ripeness levels. It will seems that in our study the CM treatment has no effect on total anthocyanin at either the farms, cultivars and ripeness levels. The enzyme treatment shows significant higher concentrations of tannins and anthocyanins than the other treatments.

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# **Chapter 4**

## **Research results**

**The influence of different winemaking techniques on the mouth feel of Shiraz grapes**

### 4.1 ABSTRACT

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The objective of the study was to determine the effect of ripeness and of different tannin extraction methods on the sensory properties of wine, with specific focus on mouth feel properties. Quantitative descriptive analysis (QDA) was performed to evaluate the sensory properties of twenty young Shiraz wines in two phases. In Phase 1, wines from a cool area were evaluated and in Phase 2, wines from a warm area were evaluated. Clear differences were found between the wines from the two regions. Wines from the cooler region were generally associated with higher levels of total non-flavonoids and total anthocyanins and more intense numbing and puckering sensations. In contrast, the wines from the warmer region as a group was associated with a more drying and grippy mouth feel as well as less total anthocyanins and total non-flavonoids. In the set of wines from the cooler region, the effect of ripeness was more pronounced than in the set of wines from the warmer region. In both cases riper grapes resulted in a coarser surface smoothness, a more numbing sensation, bitter aftertaste and less adhesive mouth feel. The wines from the cooler region that were harvested at a riper stage were associated with many of the anthocyanins/anthocyanin derivatives and were negatively associated with hydroxycinnamate, procyanidin B1 and delphinidin-3-glucoside-*p*-coumaric acid. In the warmer area, the riper grapes were again associated with anthocyanins/anthocyanin derivatives, but were this time strongly associated with procyanidin B2, caffeic acid, *p*-coumaric acid, catechin, coumaric acid and total non-flavonoids. The effect of tannin extraction method on the sensory properties of the wines from the warmer region was more pronounced than in the wines from the cooler region. Unfortunately, the differentiation between treatments was not consistent from one ripeness level to another. However, it appeared the cold soak treatment differ the least from the control regardless of region or ripeness, whereas the post maceration treatment differed the most based on mouth feel and phenolic composition. Although some mouth feel attributes and phenolic compounds were consistently associated with region and ripeness, it is not clear if mouth feel can be consistently manipulated by tannin extraction methods.

## 4.2 INTRODUCTION

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The macromolecular fraction of red wines is mainly composed of polysaccharides and polyphenolic compounds like proanthocyanidins and anthocyanidins (Vidal *et al.*, 2004a). It has been suggested that anthocyanins could modulate the astringency perception in red wines either directly or through reactions with proanthocyanidins (Gawel 1998; Vidal *et al.*, 2004a; Gawel *et al.*, 2007; Oberholster *et al.*, 2009). Brossaud *et al.* (2001) observed that an anthocyanin fraction complemented grape proanthocyanidin astringency and did not contribute to bitterness (Vidal *et al.*, 2004b). Astringency is a tactile sensation, which can be described in sensory terms as drying (the lack of lubrication or moistness resulting in friction between oral surfaces), roughing (the unsmooth texture in the oral cavity marked by inequalities, ridges and/or projections felt when oral surfaces come in contact with one another) or puckering (the drawing or tightening sensation felt in the mouth, lips and/or cheeks) and is ascribed to the binding and precipitation of the salivary proteins (Gawel *et al.*, 2001; Vidal *et al.*, 2004b; Landon *et al.*, 2008). It was found that seed tannins were more astringent (coarse, drying) than skin tannins of equivalent size, which were probably due to gallic acid derivatives (Oberholster *et al.*, 2009).

Gawel *et al.* (2001) describes the tactile sensation of astringency as follows: *“is a result from the cross-linking of polyphenols with glycoproteins found between and above the epidermal cells of the mucosal tissue in the mouth and/or from the binding and subsequent precipitation of salivary proteins by polyphenols. The polyphenol-protein interaction results in a saliva with poorer lubricating properties and greater friction between mouth surfaces. The increased friction ultimately activates the mechanoreceptors in the mouth leading to the perception of astringency.”* Therefore astringency is a characteristic of unripe fruit (Vidal *et al.*, 2004b). Astringency may be intensely perceived in young red wines but it will gradually decrease during maturation (Vidal *et al.*, 2004b).

Different other molecules influence the perception of astringency or bitterness like polysaccharides which are responsible for “mellowness” and viscosity (Vidal *et al.*, 2004a), acidity which can contribute to astringency by increasing the efficacy of bonding of polyphenols to salivary proteins (Gawel *et al.*, 2001), alcohol which can reduce the astringency sensation (Gawel *et al.*, 2001.; Fontoin *et al.*, 2008). Gawel (1998) warned

that astringency increases upon repeated ingestion with the rate of increase being greater when the time between ingestions is shortened. Lee and Vickers (2009) also find the astringent feeling can take as short as 15 seconds to fully develop (and can linger to over 6 min.) and is known to build in intensity and became increasingly difficult to clear from the mouth over repeated exposures.

The aim of this study was to investigate the mouth feel properties of Shiraz wine and the representative chemical components. We also wanted to see if there was discrimination between Shiraz wine made in two different climatic areas and of two different ripeness levels and their mouth feel properties. The outcome of this study could cast some light on the mouth feel of wine made by different winemaking techniques.

## 4.3 MATERIALS AND METHODS

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### 4.3.1 SAMPLES

#### *Grapes*

The Shiraz grapes were harvested in two climatic areas and at two different ripeness levels. The first ripeness level was before commercial harvest and the second ripeness level was after commercial harvest. The first area is classified as IV according to the Winkler scale (1965) i.e. a warm area. This area is in the Stellenbosch district of Simondium on the farm Plaisir de Merle. The second area is classified as III according to the Winkler scale (1965), therefore it is regarded as slightly cooler than the first area. This second area is located in the Durbanville district on the farm Morgenster. The Winkler heat summation works as follows: The sum of the average daily temperature above 10°C for the growth months (September to March) are calculated and are then expressed as degree days. These degree days are then compared to a table (like Table 4.1). This table can then be used to calculate which cultivar would suite the specific area.

**Table 4.1** Winkler heat summation adapted for South African climate

Area	Degree days	Potential for viticulture
I	<1389	Early cultivars, high quality, no mass production
II	1389 – 1667	High quality white and red table wine
III	1668 – 1944	Late cultivars, high quality red
IV	1945 – 2222	Natural sweet cultivars, medium quality red & white
V	>2222	Mass production, late cultivars, dessert wines

## ***Wine***

Four different winemaking techniques (CM – cold maceration, E – enzyme, PM – post maceration and CM+PM – a combination of cold and post maceration) were used to extract the tannins and anthocyanins. These winemaking techniques were evaluated against a control. The cold maceration took place for three days at 15°C and with the post maceration the skins were left for two weeks after alcoholic fermentation.

Two different tannin precipitation essays were used to quantify the tannin concentration of the Shiraz wine. The methylcellulose (MCP) assay uses a polysaccharide to precipitate tannin and is a more direct method as it is read with a spectrophotometer at 280 nm. The bovine serum albumin (BSA) assay uses a protein to precipitates tannin and is a more indirect method as it is read by a spectrophotometer at 520 nm. This method also took in consideration the bleaching effect of bisulphite. This method is therefore usefull to follow the evolution of the monopigmenst (MP), small polymeric pigments (SPP) and the large polymeric pigments (LPP) as the wine matures.

## ***RP-HPLC***

Monomeric phenolic compounds were determined in duplicate using the RP HPLC method of Donovan et al. (1998). A Hewlett-Packard/Agilent model 1100 HPLC (Palo Alto, CA) with a diode array UV-visible detector coupled to HPCHEMStation software was used. The column was a Ascentis ® Express C18 (15 cm x 4.6 mm; 2.7 µm) (Supelco, Sigma Aldrich). All wine samples and standards were filtered through 0.45-µm PTFE syringetip filters (Gelman Sciences, Ann Arbor, MI) before use. The following standards were used: gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid, and rutin (Sigma, St. Louis, MO) and malvidin-3-glucoside (Mv-3-glc) (Extrasynthese, Genay, France). Compounds were identified by group on the basis of their UV spectra and reported in terms of the related standard compound. These were benzoic acids, hydroxycinnamates, flavan-3-ols, flavonols, and anthocyanins expressed as mg gallic acid equivalents/L (mg GAE/L), mg caffeic acid equivalents/L (mg CAE/L), mg catechin equivalents (mg CE/L), mg rutin equivalents/L (mg RE/L), and mg Mv-3-glc equivalents/L (mg ME/L), respectively. The total area under the chromatograms at 280 nm were integrated and used to calculate the total phenol content expressed as mg GAE/L, probably lower than actual because of the high absorbance of gallic acid.

## Tasting

This study was conducted in two phases consisting of ten samples each as indicated in Table 4.2. The wines were harvested at two different ripening levels and were collected from Morgenster farm and Plaisir de Merle farm respectively. Five different tannin extraction procedures were evaluated within each ripening level. The area, bottling codes, treatment names and abbreviations are shown in Table 4.2. The month indicated in Table 4.2 refers to the month in which the grapes were harvested.

**Table 4.2:** List of samples evaluated in this study

Phase	Area	Month	Treatment	Bottling code	Abbreviation
1	Morgenster	March	Control	240303	C <sup>1</sup> _C <sup>2</sup> _E <sup>3</sup>
1	Morgenster	March	Enzyme	240304	C_E_E
1	Morgenster	March	Cold Soak	240305	C_CS_E
1	Morgenster	March	Post Maceration	240306	C_PM_E
1	Morgenster	March	Cold Soak and Post Maceration	240307	C_CP_E
1	Morgenster	April	Control	140406	C_C_L
1	Morgenster	April	Enzyme	140407	C_E_L
1	Morgenster	April	Cold Soak	140408	C_CS_L
1	Morgenster	April	Post Maceration	140409	C_PM_L
1	Morgenster	April	Cold Soak and Post Maceration	140410	C_CP_L
2	Plaisir de Merle	February	Control	180208	W_C_E
2	Plaisir de Merle	February	Enzyme	180209	W_E_E
2	Plaisir de Merle	February	Cold Soak	180210	W_CS_E
2	Plaisir de Merle	February	Post Maceration	180211	W_PM_E
2	Plaisir de Merle	February	Cold Soak and Post Maceration	180212	W_CP_E
2	Plaisir de Merle	March	Control	020311	W_C_L
2	Plaisir de Merle	March	Enzyme	020312	W_E_L
2	Plaisir de Merle	March	Cold Soak	020313	W_CS_L
2	Plaisir de Merle	March	Post Maceration	020314	W_PM_L
2	Plaisir de Merle	March	Cold Soak and Post Maceration	020315	W_CP_L

<sup>1</sup> Refers to climatic region (C=Cool area, W=Warm area)

<sup>2</sup> Refers to the treatment name (C=Control; E=Enzyme; CS=Cold soak; PM= Post maceration; CP=Cold soak and post maceration)

<sup>3</sup> Refers to the harvest date (E=Early, L=Late)

### **4.3.2 SENSORY METHODOLOGY**

Quantitative descriptive analysis with a trained panel was used following the procedures outlined in Lawless and Heymann (1995). The same experimental design, testing facilities and sample preparation/presentation procedures were used in Phase 1 and Phase 2.

### **4.3.3 PANEL**

Eleven panellists were selected to participate in the profiling of the wines. Eight of the panellists had previous experience in quantitative descriptive analysis. The remaining three panellists were selected based on taste and smell acuity, interest, ability to discriminate between the four basic tastes, ability to verbally describe sensory experiences, concentration abilities and availability. One of the panellists could not attend the second phase of the project, and therefore Phase 2 was conducted with only ten panellists.

### **4.3.4 TRAINING**

In the first training phase the panel evaluated the ten samples from Morgenster. The panel was trained for 8 weeks (2 x 2hr sessions per week) during which the panellists received representative samples of the different wines and were trained to increase their sensitivity and ability to discriminate between specific samples and the sensory attributes of each product sample. The list of sensory attributes with representative reference standards is shown in Table 4.3. Aroma identification guides, using the definitions of each descriptor, were compiled to help the panellists identify aromas, flavours and mouth feel properties during tasting based on an elimination process (Addenda A-C). Throughout the training, the panellists were given aroma reference standards representing the aroma attribute term and ask to identify the aromas of each reference standard on a blind basis. The panellists were also provided with touch and taste standards for the mouth feel terms as indicated in Table 4.3.

The panel used a 100 mm line scale, with nil (0) denoting the least intense condition (e.g. no fresh berry aroma) and hundred (100) denoting the most intense condition (e.g. intense fresh berry aroma) to evaluate the aroma, flavour, aftertaste and mouth feel

characteristics of the different products. The attributes 'particulate/graininess' and 'surface smoothness' were evaluated on a 100mm scale where nil (0) denoted the most smooth condition and hundred (100) denoted the most coarse condition. During the training phase the panel performance was monitored using Tucker plots, profile plots, and three-way analysis of variance using the Panel CHECK 1.3 software ([www.panelcheck.com](http://www.panelcheck.com)).

The same training protocol was used during Phase 2 (evaluation of Plaisir de Merle wines). The different list of aroma and flavour attributes, that was more representative of the characteristics of the Plaisir de Merle wines, was used during Phase 2 (Addendum D). The mouth feel and aftertaste attributes were the same.

#### **4.3.5 EXPERIMENTAL DESIGN USED DURING FINAL SAMPLE EVALUATION**

Quantitative descriptive analysis was conducted over a three day period in order to incorporate three replications. Each taster evaluated eight samples in a balanced incomplete block design.

#### **4.3.6 TEST FACILITIES**

The sensory descriptive test was conducted in Distell's Sensory Laboratory equipped with 12 separate booths designed according to American Society for Testing Materials (ASTM, 1989) standard requirements. Panellists evaluated products monadically in separate tasting booths to reduce distraction and panellist interaction and to ensure uninterrupted, unbiased, individual responses. Data were collected using the computerized data collection software Compusense five Release 5.0. (Compusense Inc., Guelph, Canada).

#### **4.3.7 SAMPLE PRESENTATION**

The wine samples were served according to research guidelines for the sensory evaluation measurements of alcoholic beverages. All samples were randomized to exclude any bias due to the position effect. The samples were served monadically at room temperature. Two 15 ml samples were served per wine in Vitria ISO tasting glass, covered with a Petri dish and coded with a three digit random number. The panellists

were instructed to taste the entire 15 ml of the first glass for their evaluation. This was done to reduce any variation in mouth feel evaluations due to variance in sip sizes. The second glass was provided in case they wanted to re-evaluate some of the flavour attributes. Between samples, panellists were instructed to cleanse their pallets with distilled water and unsalted water crackers served at room temperature. A time delay of three minutes was incorporated between samples. After rating four samples in this manner, a ten minute break was introduced in order to avoid sensory adaption. Each panellist was provided with sensory attribute identification guide during the evaluation of the products.

**Table 4.3:** List of reference standards used to illustrate the respective sensory attributes measured in this study. All aroma standards are presented in a neutral red wine

Attribute	Definition	Reference	Dosage
Fresh/Ripe fruit	Fresh, tart, lively aroma/flavour associated with fresh berry fruit	Mixture of frozen strawberries, raspberries, blackberries	400 g/L
Unripe/green fruit	Sour and/or slight green aroma/flavour note associated with fruits not yet ready for eating	No reference	
Overripe fruit/Jammy	Cooked, thick, syrupy note associated with fruit jam	Mixed fruit jam (100ml)	500ml/L
Cordial	A sweet, confectionary, 'Cool-Aid' character	Raspberry (Sensient)	0.5ml/L
Fresh veg/leafy/herbal	Sharp vegetative notes associated with grass, fresh herbs and green stalks.	Grassy (IFF 00022010) Eucalyptus (Burgess and Finch) Fresh flat leaf parsley	0.4 ml/L 0.2 ml/L 40g/L
Canned/cooked Veg	Slightly sweet and stuffy aroma/flavour note associated with canned vegetables in a wine	1:1:1 Mixture of the brine of canned asparagus, canned green beans and canned garden peas	200 ml
Sulphury	An aroma and flavour note associated with sulphur a compound that is reminiscent of a mushroom farm.	No reference	
Stuffy	Mouldy earthy character associated with wet straw, compost, cheese. Not a fresh character. Unpleasant character.	Blue cheese 2,4,6-trichloroanisole (1mg/L stock)	2% 4 ng/L
Savoury Veg	A sweet-savoury note with a vegetative character – malty, hay, straw, soy sauce	Herbaceous Malt2 (IFF) Herbaceous Tea (IFF) Soy Sauce (Vital)	0.25 ml/L 0.5 ml/L 20 ml/L
Earthy	Pleasant, comforting, natural aroma associated with garden/potting soil	Earthy (Firminich)	0.5 ml/L
Particulate	Feelings of particulate matter brushing against the surfaces of the mouth through the movement of the wine.	Maizena Corn Starch (Fine)	As is
		Fine Bentonite powder (Medium)	As is
		Icing sugar (Medium)	As is
		Course Bentonite powder (Course)	As is
		Sifted Whole wheat flour (coarse fraction that remains in the seive) (Course)	As is

**Table 4.3: (cont.)**

Attribute	Definition	Reference	Dosage
Surface Smoothness	Smoothness of mouth surfaces when the different surfaces (tongue and cheek) come in contact with each other.	Satin cloth (Fine)	As is
		Velvet cloth (Medium)	As is
		1000 grade sandpaper (Medium)	As is
		Corduroy (Course)	As is
		600 grade sandpaper (Course)	As is
Grippy	Distinct lack of slip between mouth surfaces resulting in the inability to easily move mouth surfaces across each other. Slightly abrasive sensation in inner mouth cavity	Tannin VR Supra	2.5 g/L
Adhesive	The feeling that mouth surfaces are sticking or adhering to one another, yet can be pulled away from each other with slight pressure.	Alum	0.8 g/L
Pucker	Sensation that cheeks are drawn towards each other. Tightening sensation.	No reference	
Drying	Loss of lubrication in oral cavity. Impression of thirstiness	No reference	
Numbing	Slight loss of sensation in oral cavity. Swollen feeling of oral tissues	No reference	
Sour	Basic taste sensation on the tongue that is caused by acids	Citric acid	0.7 g/L
Bitter	Sharp basic taste sensation at the back of the tongue caused by stimuli such as aloe, caffeine and aspirin	Caffeine	0.75 g/L

(Adapted from Gawel *et al.*, 2000)

#### **4.3.8 DATA ANALYSIS**

The scale values obtained for the attribute 'surface smoothness' was inverted so that low ratings refer to a coarser mouth feel and high ratings refer to a smoother mouth feel. Three-way analysis of variance (ANOVA) was performed to determine if there were significant judge\*sample or sample\*replication interactions and whether there were significant differences between the samples. Fishers' LSD post hoc tests were performed to determine which samples differed significantly from which. Principal component analysis (PCA) was performed to obtain a graphical representation of the interrelationships of the sensory attributes and/or phenolic composition of the samples. It was applied on the mean values of the attributes and phenolic compounds per sample. The data was centered and standardized prior to the PCA analysis. Agglomerative hierarchical cluster analysis (AHC) was performed to further investigate the relationships between the sensory and chemical variables. ANOVA, PCA and AHC procedures were performed in XLStat version 2009.1.02 (Addinsoft, [www.xlstat.com](http://www.xlstat.com)). .

#### **4.4 RESULTS AND DISCUSSION**

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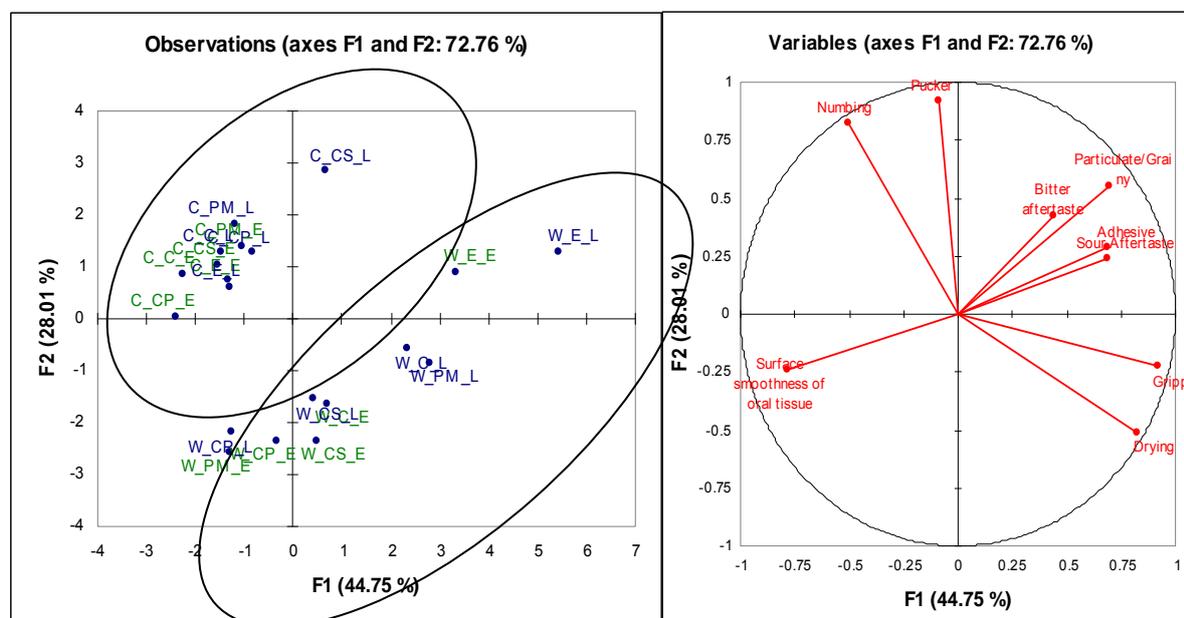
The results obtained from the analysis of variance (ANOVA) for the 10 wine samples evaluated in Phase 1 and Phase 2 is summarized in Table 4.7 and Table 4.8 at the end of the document. The mean values for all samples are provided and the statistical significance of the differences ( $p \leq 0.05$ ) is indicated. The univariate results will be discussed as supporting data for the interpretation of the multivariate analysis.

##### **4.4.1 THE EFFECT OF CLIMATIC AREA ON MOUTH FEEL AND PHENOLIC COMPOSITION OF RED WINE**

A PCA on the mouth feel attributes of all twenty samples was done to investigate the effect of climatic region, ripeness level and tannin extraction method on the mouth feel of red wines (Figure 4.1). The first two principal components (PC) explained 72.8% of variance in the dataset (Table 4.4). The scores plot shows a clear differentiation between the samples from the warmer area (denoted by W) and the samples from the cooler area (Denoted by C). All the samples from the cooler area had positive scores on PC 2 (i.e. situated at the top of the plot). With the exception of the enzyme treatments, all the samples from the warmer region had negative scores on PC2.

**Table 4.4:** Cumulative % variance explained by PCA's done on various combinations of mouth feel and chemical properties of all twenty wines

Sample set	F1	F2	F3
Mouth feel	44.750	72.760	84.458
Chemistry	44.873	60.220	72.195
Mouth feel and major chemical groups	37.910	67.394	77.587

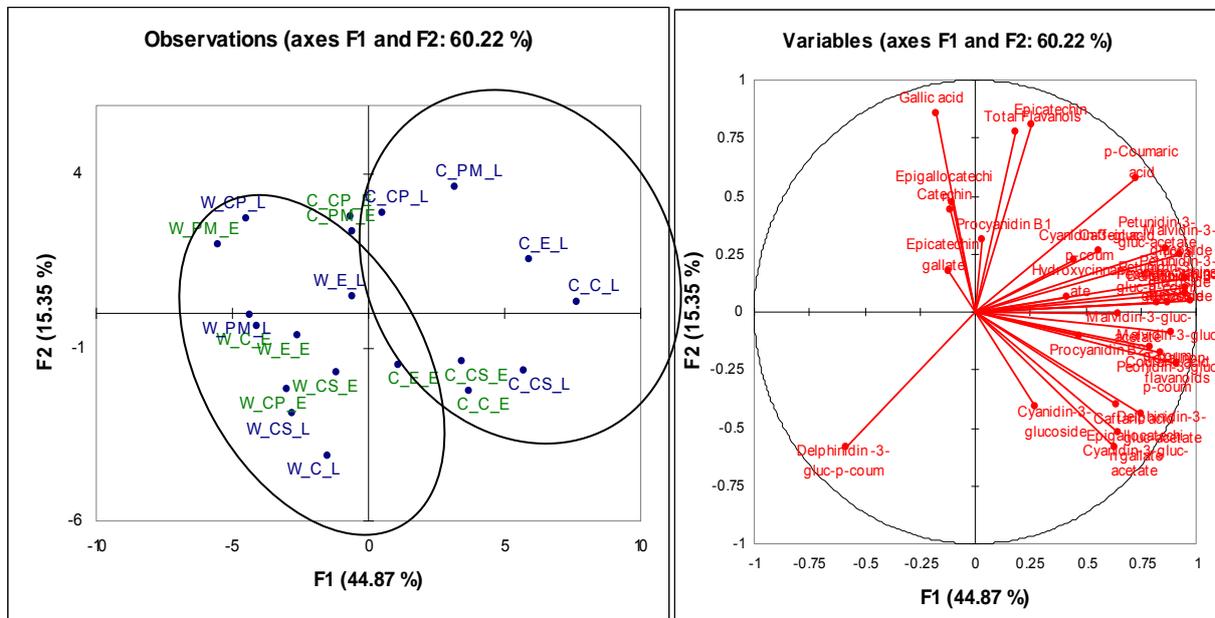


**Figure 4.1:** PCA showing the differentiation between samples from a warm and cooler area based on mouth feel attributes

The attributes puckering and numbing were strongly associated with PC 2. Overall the wines from the cooler area (Table 4.7) were deemed to cause a more intense numbing and puckering effect compared to the wines from the warmer area (Table 4.8). The attributes, grippy and drying had the strongest negative association with PC 2 (i.e. associated with the wines from the warmer climate). A closer look at the univariate results confirms that the wines from Plaisir de Merle were overall rated as more grippy and drying than the wines from Morgenster. This PCA did not show a clear trend in terms of the ripeness levels (marked as blue and green) or treatments within in each region.

A second PCA was performed on the phenolic composition of the wines (Figure 4.2). This PCA explained only 60% of the total variance in the data (Table 4.4). In Figure 4.2, one can see that the degree of differentiation between the samples from the warmer (W) and cooler (C) area was not as clear based on the phenolic composition compared to the sensory attributes in Figure 4.1. There was still a fair degree of separation between the W samples and the C samples as indicated by the two circles.

Based on the variable loadings (Figure 4.2), delphinidin-3-glucoside-*p*-coumaric acid had the strongest association with the W samples. This compound had a strong negative association with *p*-coumaric acid (which was associated with the C samples). One could hypothesise that the warmer growth conditions encouraged the binding process of *p*-coumaric acid and delphinidin-3-glucoside.

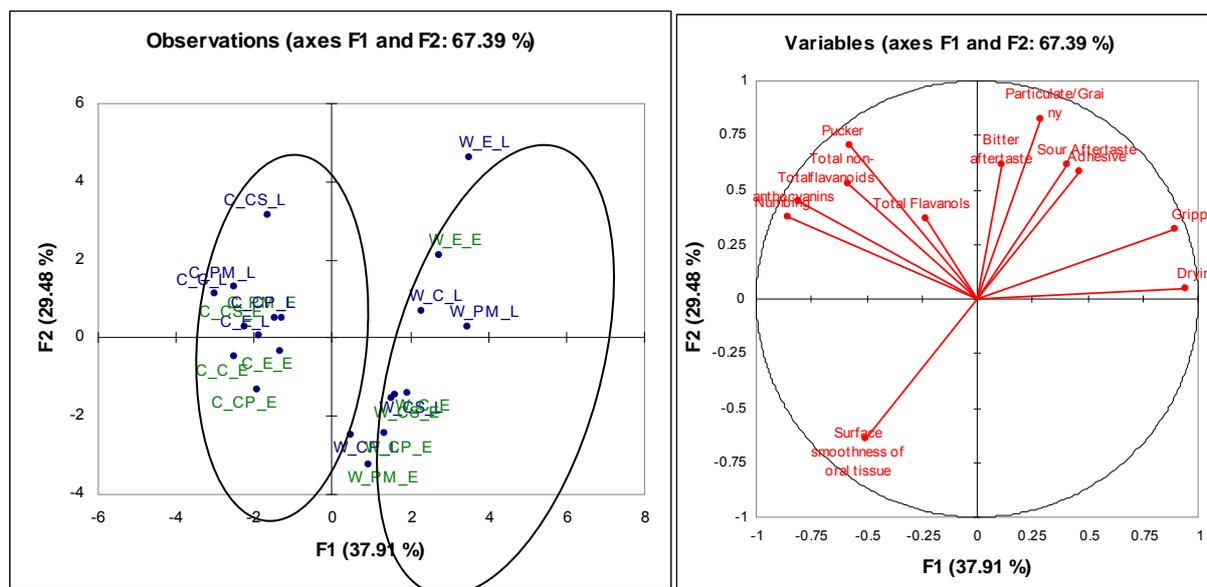


**Figure 4.2:** A PCA on the phenolic composition shows some differentiation between samples from a warm and from a cooler region respectively

A PCA on the mouth feel and major chemical groupings (e.g. total flavanols) revealed even more interesting results (Figure 4.3). As shown in Table 4.2, the first two PC's explained 67% of the variance in the data. PC 1 showed a clear separation between the samples from the warmer climatic region (W) and the wines from a cooler climatic region (C). PC 1 had a positive correlation with drying and grippy, and was negatively correlated with numbing, puckering, total non-flavonoids and total anthocyanins. This means that the wines from the cooler region was generally associated with higher levels of total non-flavonoids and total anthocyanins and more intense numbing and puckering sensations. In contrast, the wines from the warmer region as a group was associated with a more drying and grippy mouth feel as well as less total anthocyanins and total non-flavonoids.

In addition to the differentiation between the regions, this PCA also showed some differences between the wines that were harvested early (green) vs. the wines that were harvested later (blue) on PC 2 (Figure 4.3). The wines that were harvested earlier were generally associated with a finer surface smoothness, while wines that were harvested

later were associated with a more particulate/grainy mouth feel as well as a more bitter aftertaste. Although the separation between the ripeness levels were represented more clearly in this PCA, the chemical parameters did not significantly contribute to the differentiation observed. The effect of ripeness will be evaluated in more detail within the wines from Plaisir de Merle and the wines from Morgenster respectively.



**Figure 4.3:** PCA scores and loadings plot showing the differentiation of samples based on mouth feel and phenolic properties

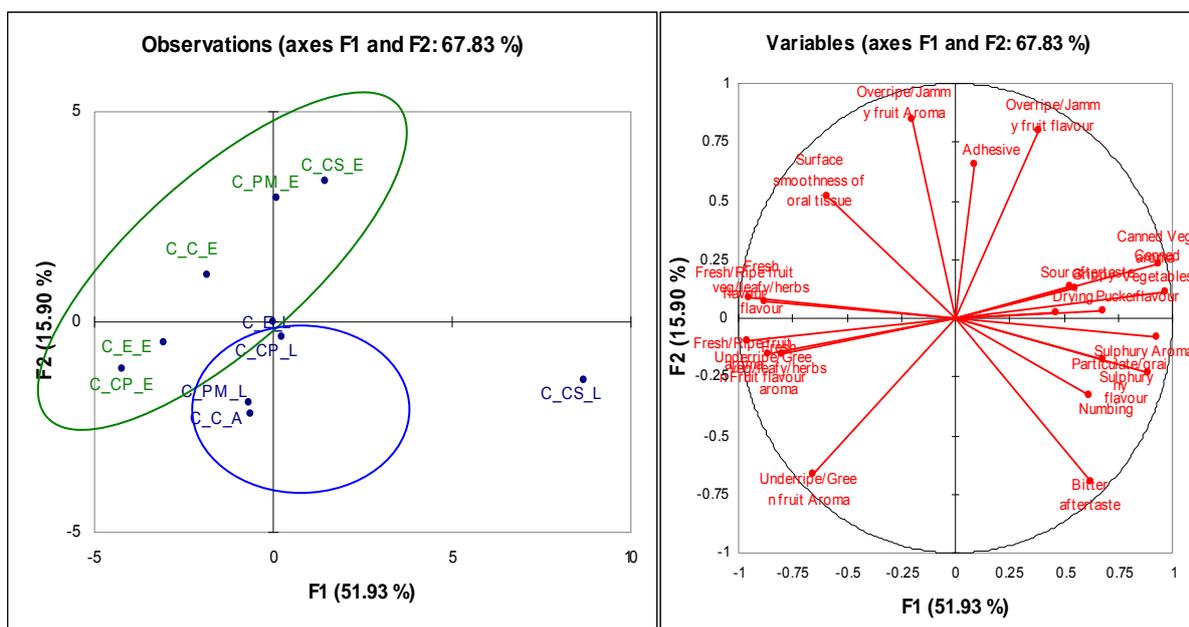
#### 4.4.2 OVERALL EFFECT OF RIPENESS LEVEL ON THE SENSORY ATTRIBUTES AND PHENOLIC COMPOSITION OF WINES HARVESTED IN A COOL AREA

A PCA was done on the aroma, flavour, taste and mouth feel properties of the wines evaluated in Phase 1 of the project (cool area). According to the results of the PCA, the first principal component (PC 1) accounted for 51.9% of the total variance in the data (Table 4.5). The second principal component accounted for 15.9% of the total variance. PC 3 accounted for a further 11.1% of the variance in the data. A total of 78.9% of the data could be explained by the first three PC's.

**Table 4.5:** Percentage cumulative variance explained by PCA's on the wines from a cool climatic region

	F1	F2	F3
Sensory data (Figure 4.6)	51.9	67.8	78.9
Sensory data without C_CS_L (Figure 4.5)	42.3	60.9	74.9
Sensory and chemical data (Figure 4.7)	34.9	61.3	73.8

C\_CS\_L was a clear outlier in the sample set (Figure 4.4). This sample differentiated from all the others based on significantly more intense cooked/canned vegetables aroma and flavour and sulphury aroma and flavour (Table 4.7). C\_CS\_E had the second highest intensity of these four characteristics, although in each case at significantly lower intensities than C\_CS\_L. Canned/cooked vegetable aroma and flavour occurred at very low intensities in the other wines. C\_CS\_E had a low, but noticeable sulphury aroma, but this characteristic disappeared on flavour. In the case of C\_CS\_L, the sulphury characteristic also decreased in intensity from aroma to flavour, but was present at medium to medium-high intensities. The intensity of the sulphury aroma and flavour in the other wines were negligible. In addition to the canned/cooked vegetable and sulphury aromas and flavours, C\_CS\_L also differentiated from the other wines in terms of mouth feel properties. The position of C\_CS\_L on the scores plot is further associated with pucker, numbing and bitter aftertaste. C\_CS\_L induced a more intense puckering sensation than C\_CP\_E and C\_CP\_L. C\_CS\_L also caused a more intense numbing sensation than C\_C\_E. Furthermore, C\_CS\_L had a significantly more bitter aftertaste than C\_C\_E, C\_E\_E, C\_CS\_E, C\_PM\_E, C\_CP\_E as well as C\_E\_L.

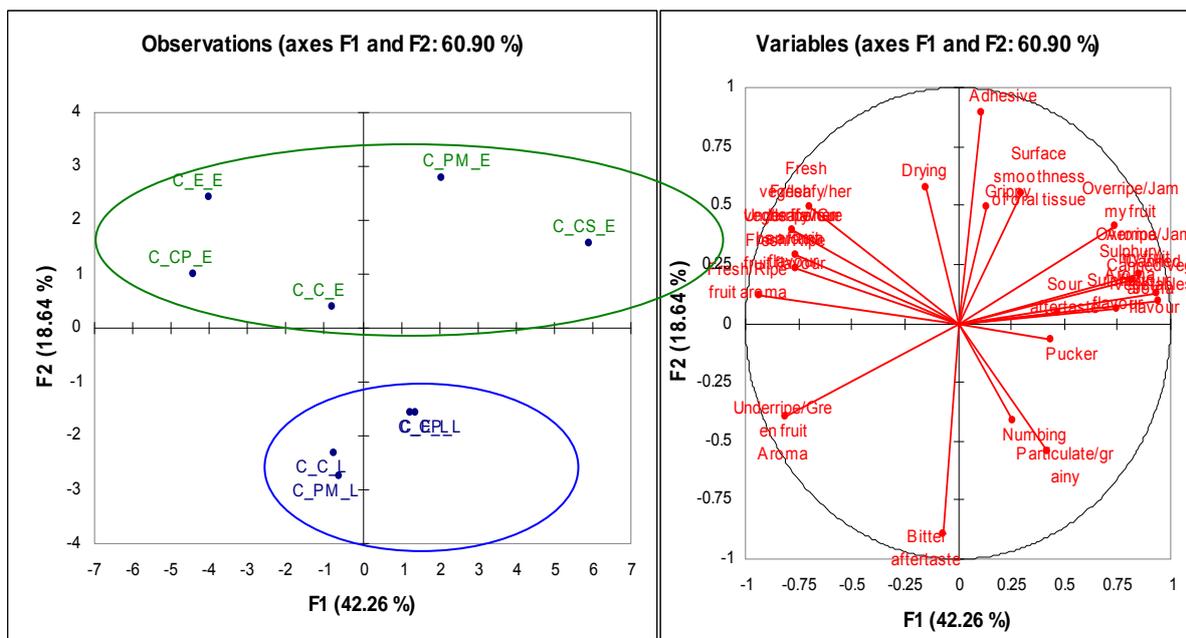


**Figure 4.4:** PCA scores and loadings plot showing the differentiation among wines from a cool climatic region based on their sensory attributes. Wines highlighted in green were harvested earlier than the wines highlighted in blue.

In order to visualize the differentiation between the two ripeness levels more clearly it was decided to regard C\_CS\_L as an outlier. A PCA was performed where C\_CS\_L was excluded from the analyses. The total variance explained (60.9%) is shown in Table

4.5. On the PCA scores plot (Figure 4.5), the wines from the two ripeness levels are separated on PC 2. The samples that were harvested earlier had positive loadings on PC 2, while the samples harvested later had negative loadings on PC 2. The loadings plot shows that the differentiation between the two ripeness levels are driven by mouth feel attributes rather than aroma and flavour attributes. As shown in the loadings plot, the attributes 'adhesive' and 'bitter aftertaste' were the most influential contributors to the differentiation between the samples on the PC 2 axis. According to the ANOVA results (Table 4.7), the early samples were more adhesive as a whole. However, only C\_PM\_E and C\_PM\_L differed on a statistically significant level from each other in this attribute. Similarly, the riper samples were all more bitter than the samples that were harvested earlier, but only C\_C\_E and C\_C\_L differed at a statistically significant level. Although C\_CS\_L was excluded from the PCA, the ANOVA results showed that this sample was significantly more bitter than C\_CS\_E. They did not differ significantly in terms of adhesiveness.

In addition to these two attributes, grippy, surface smoothness and numbing also contributed to the differentiation between ripeness levels. There was a trend that the earlier harvested samples was more grippy and had a finer surface smoothness overall, whereas the riper samples were generally more numbing.



**Figure 4.5:** Differentiation between two ripeness levels of wines harvested in a cool region based on sensory attributes on a PCA scores and loadings plot.

#### **4.4.3 THE EFFECT OF TANNIN ON THE SENSORY ATTRIBUTES OF WINES IN A COOL AREA**

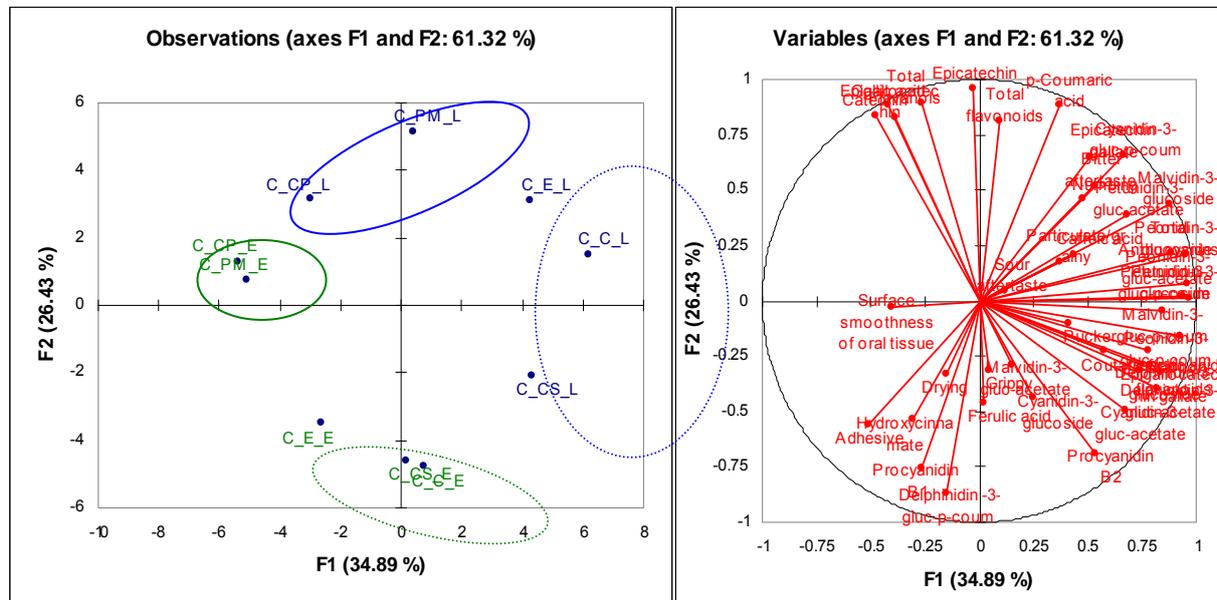
The differentiation between the tannin extraction treatments were shown more clearly on the second and third PC's of a PCA done on the sensory attributes of the wines (Figure 4.6). The wines from the lower ripeness levels are circled in green in Figure 4.6 and the wines from the riper ripeness level are circled in blue. In both cases, the control and cold soak wines were the most similar and separated from the other wines in the respective ripeness level on PC 3.

In the case of the wine that were harvested earlier, the C and CS treatments differentiated the most from the E and PM treatments, while CP could be seen as an intermediate. The E and PM wines were both more drying and adhesive than the C and CS wines (positive association with PC 3). CP were more drying but not more adhesive than C and CS. C and CS were slightly more particulate/grainy but not at a statistically significant level.

In contrast, the C and CS treatments of the riper group of wines were more drying than PM and E. The CS treatment was also more adhesive than PM and E. Furthermore, CS and C were more sour than PM and E. Therefore, although the treatments had a significant effect on the sensory properties of the wines, the way in which they differed changed as the ripeness level increased.



coumaric acid, where the former was associated with grapes from a cooler climate and the latter with grapes from a warmer climate. In this case, delphinidin-3-glucoside-*p*-coumaric acid was associated with grapes that were harvested at a lower ripeness level, while *p*-coumaric acid was associated with grapes that were harvested at a riper stage.



**Figure 4.7:** PCA scores and loadings plot showing the differentiation between ripeness levels and winemaking treatments based on mouth feel attributes and phenolic composition.

#### 4.4.5 THE INFLUENCE OF PHENOLIC COMPOSITION ON THE DIFFERENT WINEMAKING TREATMENTS IN A COOL AREA

Figure 4.7 also shows groupings among the winemaking treatments. Within each ripeness level, there are two major sample groupings. In each case, the CP and PM treatments group together, while the E, C, and CS treatments group together. There seems to be a larger variation between treatments in the wines that were harvested later (blue) than the wines that were harvested earlier (green). On this PCA it also appears that the CS treatment was closer to the control at a lower ripeness level, while the E treatment was more similar to the control at a riper ripeness level.

The differentiation between the CP and PM treatments and the C, E and CS treatment groups are mainly driven by phenolic composition. The CP and PM treatments are associated with higher levels of total flavanols, gallic acid, epigallocatechin and catechin. The C, E and CS treatments are associated with higher levels of cyanidin-3-

glucoside-acetate, delphinidin-3-glucoside-acetate, delphinidin-3-glucoside and epigallocatechin gallate.

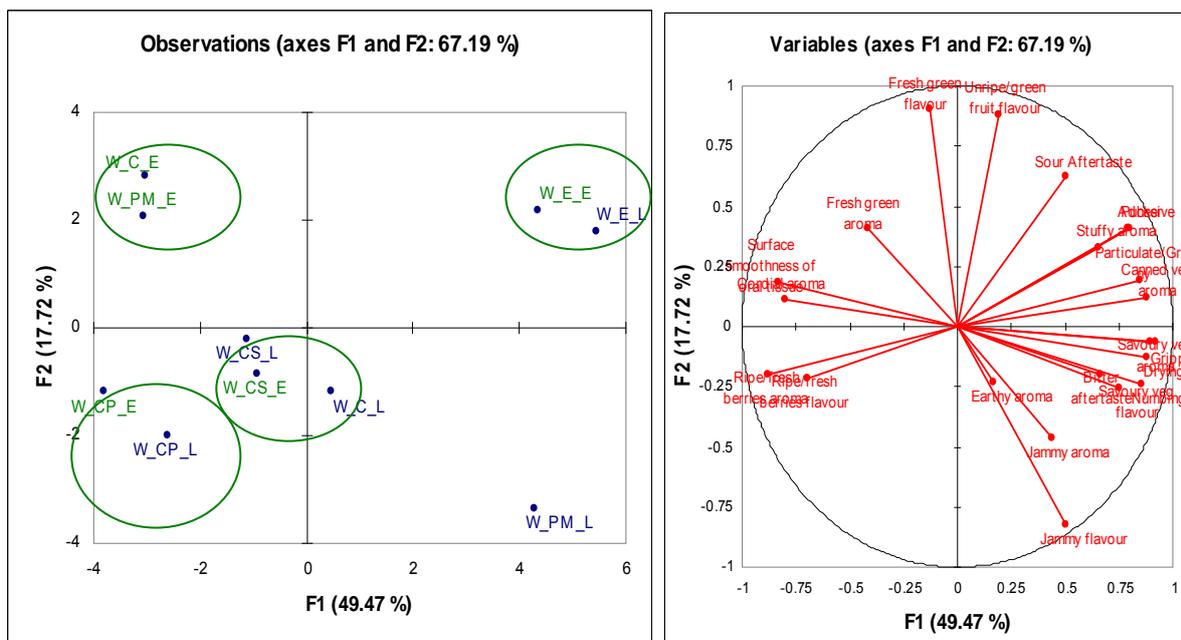
It is interesting that in terms of both sensory and chemical data the CS and C treatments appears to group together and that the PM and CP treatment always differentiates from the control. It also seems as if the perceived differences between the E and C treatments are larger than the phenolic composition suggests. The PM and CP treatment appears to have the biggest overall effect on mouth feel and phenolic composition in a cool climate.

#### 4.4.6 OVERALL EFFECT OF TANNIN AND RIPENESS LEVELS IN A WARM AREA

A PCA on the Plaisir de Merle samples from Phase 2 revealed the following trends and variations. The first two principal components explained 67.19% of the variance in the data set (Table 4.6)

**Table 4.6:** Percentage cumulative variance explained by PCA's performed on wines from a warm climate region

Data set	F1	F2	F3
Sensory data (Figure 4.8)	49.5	67.2	77.1
Mouth feel data (Figure 4.9)	68.4	82.3	90.3
Mouth feel and chemistry data (Figure 4.10)	35.5	57.3	72.6



**Figure 4.8:** The relationship between ripeness and tannin extraction methods and their effect on sensory attributes of wines from a warm climate as illustrated through PCA

As shown in Figure 4.8, the ten samples from Plaisir de Merle formed distinct groups based on their sensory characteristics. Unlike the samples from Morgenster, there were no obvious outliers. In this case, the samples grouped according to treatment effect rather than ripeness effect. The enzyme (E) treatments, cold soak (CS) treatments and cold soak and post maceration (CP) treatments formed separate three clusters. This suggests that the effect of these three treatments on the sensory characteristics of the wines were stronger than the effect of ripeness level. In contrast, the control (C) treatments and post maceration (PM) treatments had a direct negative correlation with their respective ripeness level counterparts. This suggests that the effect of these treatments on sensory properties of wine were overshadowed by the effect of ripeness in this specific sample set.

#### 4.4.7 THE OVERALL EFFECT OF TANNIN IN A WARM AREA

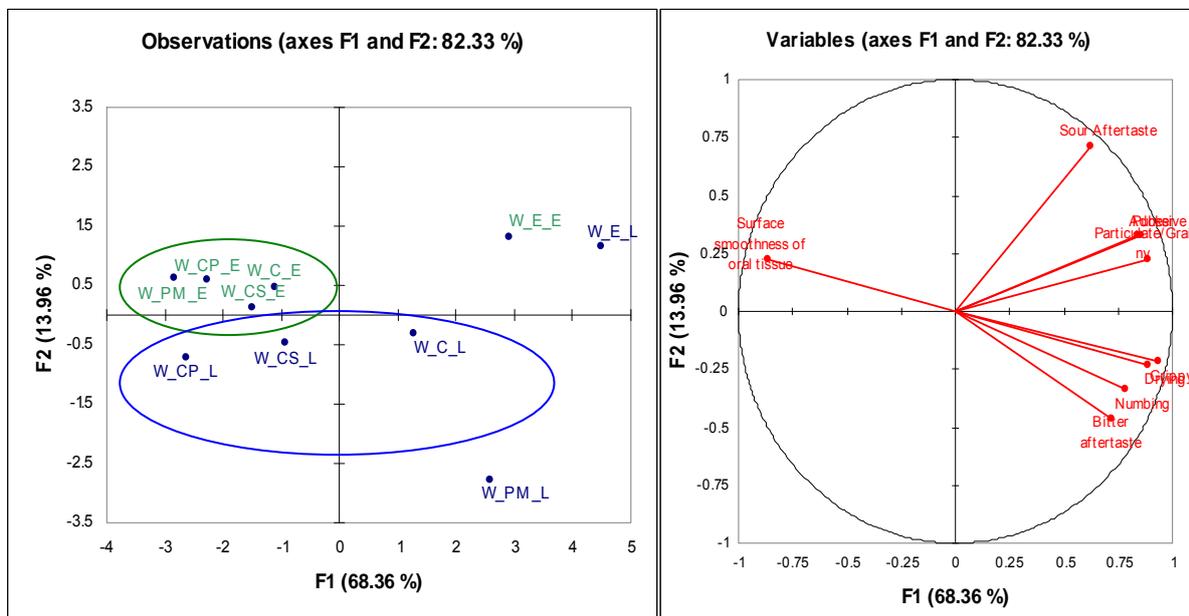
The two enzyme treatments strongly differentiated from the rest of the samples due to high positive scores on both PC1 and PC2. PC 1 was positively associated with the following attributes: canned veggies aroma, stuffy aroma, savoury veggies aroma and flavour, particulate/graininess, adhesiveness, grippy, pucker, drying, numbing and bitter aftertaste. The two enzyme treatments were significantly more intense than many of the other treatments in these attributes. The significant differences are shown in Table 4.7 and will be discussed in more detail at a later stage.

PC 1 was also negatively associated with cordial aroma, fresh berry aroma and flavour as well as surface smoothness. The two enzyme treatments had the least intense fresh berry aroma and flavour (Table 4.8). E\_E did not have any cordial notes, whereas E\_L had very weak cordial aroma notes. E\_L were one of the coarsest samples whereas E\_E were average in terms of surface smoothness.

It appears as if the effect of the PM treatment on the sensory attributes of the wines was less significant than the other treatments, especially in the wines that were harvested earlier (Figure 4.8). The PM\_L treatment differentiated more from C\_L, indicating that the effect of post maceration were more significant in riper grapes.

#### 4.4.8 THE OVERALL EFFECT OF RIPENESS ON MOUTH FEEL IN A WARM AREA

A PCA on the mouth feel properties of the ten wines from Plaisir de Merle resulted in a clearer differentiation between wines from the two respective harvest dates (Figure 4.9). The first two principal components explained 82% of the variance in the data (Table 4.6).



**Figure 4.9:** PCA scores and loadings plot showing the differentiation between samples based on mouth feel properties

The differentiation of E\_E, E\_L and PM\_L was still the most important source of variance in the data set as shown in the separation of these three products from the rest of the set on PC 1. On PC1, these samples were negatively associated with surface

smoothness, and according to the ANOVA results, E\_L and PM\_L were perceived to have the coarsest surface smoothness. All the other mouth feel attributes had strong positive loadings on PC 1 and therefore contributed to the separation of E\_E, E\_L and PM\_L from the rest of the samples. Based on the ANOVA results, these samples, together with C\_L, can collectively be regarded as the most 'astringent' overall. However, the 'astringency' of these four samples is manifested in different ways; therefore they are not clustered together on the PCA scores plot.

The differences between the harvest dates are more prominently described by PC 2. The earlier harvest date wines had positive loadings on PC 2, which was associated with the characteristics, surface smoothness, sour aftertaste, adhesiveness, pucker and particulate grainy. On the opposite end of PC 2, associated with the riper grapes, are the attributes drying, grippy, numbing and bitter aftertaste.

The treatments PM, E and C harvested earlier were significantly finer on surface smoothness than their riper counterparts. CP\_E and CP\_L did not differ significantly on surface smoothness. CS\_L were the exception where the riper grapes resulted in smoother wines. All the wines that were made from riper grapes were perceived as more numbing than their earlier harvested counterparts. These differences were significant in the cases of PM and CS, but not in the rest of the treatments. Overall the wines were not perceived as very numbing. PM\_L caused a significantly more dry sensation than PM\_E. The enzyme and control treatments were also perceived as more drying and grippy when made from riper grapes, but not at a statistically significant level. This trend was not observed in the CS and CP treatments and the differences between harvest dates of these two treatments were not significant in terms of drying or grippiness. All the wines made from riper grapes were perceived as more bitter than their less ripe counterparts. The difference was only significant for the PM treatment. The CS treatment had the second largest difference between early and later harvested grapes in terms of bitterness.

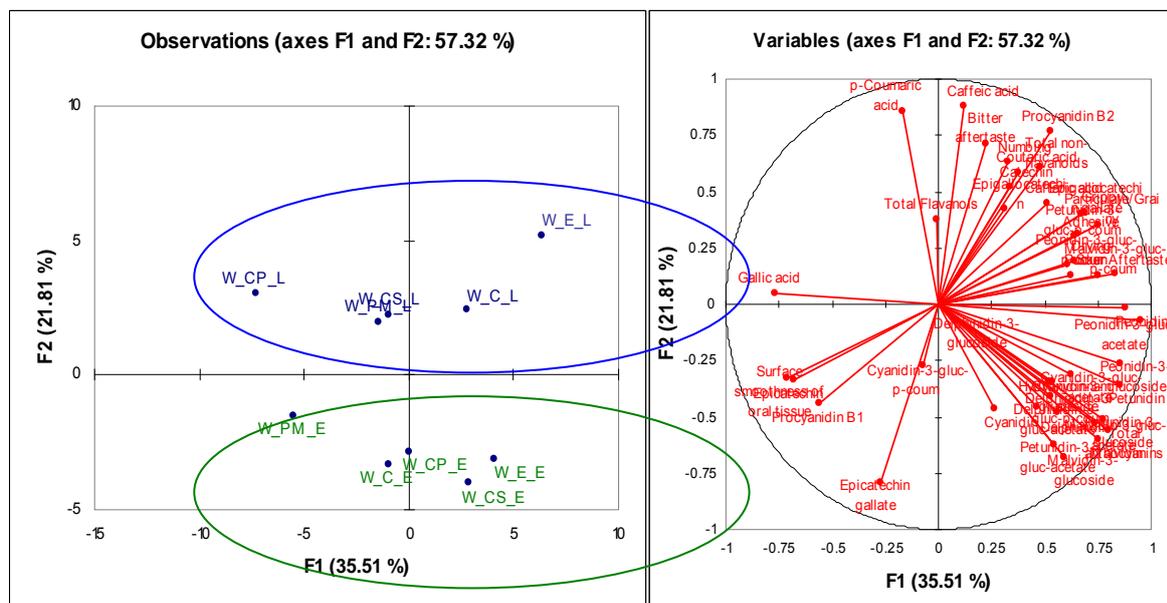
#### **4.4.9 THE INFLUENCE OF CHEMICAL COMPOSITION IN A WARM AREA**

Including the chemical parameters in the PCA increased the differentiation between harvest dates even more (Figure 4.10). A PCA done on the mouth feel and detailed phenolic composition of the wines explained 57% of the variance in the data in the first

two PC's (Table 4.6). The third PC contributed a further 15% to the total explained variance (accumulating to 73%). The differentiation between ripeness levels was explained on PC 2, where the riper grapes were positively associated and the more unripe grapes were negatively associated with PC 2.

Epicatechin gallate was strongly associated with the negative side of PC 2 and therefore with the riper grapes. Petunidin-3-glucoside, malvidin-3-glucoside, malvidin-3-glucoside-acetate and malvidin-3-glucoside-*p*-coumaric acid were also associated with riper grapes but to a lesser extent.

On the opposite end of PC 2, the grapes that were harvested earlier were associated with bitterness, procyanidin B2, caffeic acid, *p*-coumaric acid and total non-flavonoids. Numbing, catechin and coumaric acid also contributed to the differentiation on PC 2, but to a lesser extent. In this case, the inverse relationship between *p*-coumaric acid and delphinidin-3-gluc-*p*-coum was not significant.



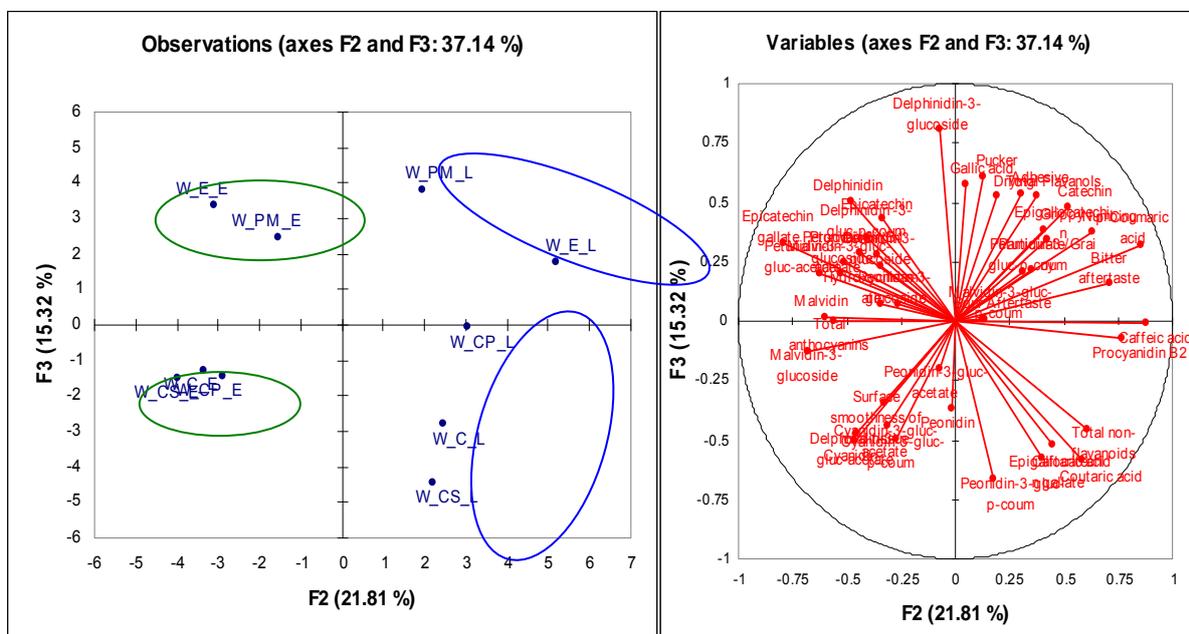
**Figure 4.10:** PCA scores and loadings plot showing the differentiation between ripeness levels based on mouth feel and phenolic composition

The third PC highlights the influence of the phenolic composition on the clustering among treatments (Figure 4.11). Within each ripeness level the E and PM treatments are positioned in one cluster, with positive scores on PC 3. The C, CS and CP treatments forms a second cluster with negative scores on PC 3 for each ripeness level. Interestingly, the clustering among the riper group less concentrated than in the less

ripe group. This may suggest that the effect of the treatments on the phenolic and mouth feel of the wines becomes more obvious as ripeness increases.

Based on the variable loadings, it appears as if the E\_E and PM\_E group is associated with the compounds delphinidin, epicatechin, delphinidin-3-glucoside-*p*-coumaric acid, epicatechin gallate and delphinidin-3-glucoside. In contrast, the CS\_E, C\_E and CP\_E cluster seems to be associated with cyanidin, cyanidin-3-glucoside-*p*-coumaric acid and cyanidin-3-glucoside-acetate. It appears as if the differentiation between the E and PM treatments from the rest of the treatments, within the early harvested group, is based on an interrelationship between delphinidin, cyanidin and their derivatives.

In the riper group, on the right side of the scores plot, the PM\_L and E\_L cluster is associated with gallic acid, catechin, total flavanols, *p*-coumaric acid and delphinidin-3-glucoside. At the opposite end of PC 3, the CS\_L, C\_L and CP\_L cluster is associated with epigallocatechin gallate, caftaric acid, coumaric acid, total non-flavonoids and peonidin-3-glucoside-*p*-coumaric acid. In this case, the differentiation between the clusters does not appear to be related to specific groups of non-flavonoids, but rather to total non-flavonoids.



**Figure 4.11:** PCA scores and loadings plot showing the differentiation between treatments based on phenolic composition and mouth feel

#### 4.4.10 THE RELATIONSHIP BETWEEN MOUTH FEEL AND PHENOLIC COMPOSITION IN A WARM AREA

Hierarchical cluster analysis (Figure 4.12) showed that the mouth feel and phenolic compounds formed three clusters. The smallest cluster consisted of procyanidin B1, gallic acid and epicatechin. These variables did not seem to have a strong relationship with any of the mouth feel attributes.

Another cluster consisted of mostly anthocyanins and anthocyanin derivatives, along with epicatechin gallate, epigallocatechin gallate, hydroxycinnamate, *p*-coumaric acid, caffeic acid and coumaric acid. The mouth feel attributes, pucker and numbing, were also associated with this group. The puckering sensation was closely associated with malvidin-3-glucoside-acetate, while the numbing sensation was associated with anthocyanins as a whole.

In the final cluster, procyanidin B2, total non-flavonoids, caffeic acid, total flavanols, epigallocatechin and catechin clusters together along with the rest of the mouth feel attributes. However, this cluster is less homogenous than the first cluster, which suggests that the relationship between numbing and puckering with the phenolic compounds in the first cluster is stronger than the relationship between the mouth feel and phenolic variables in the second cluster.

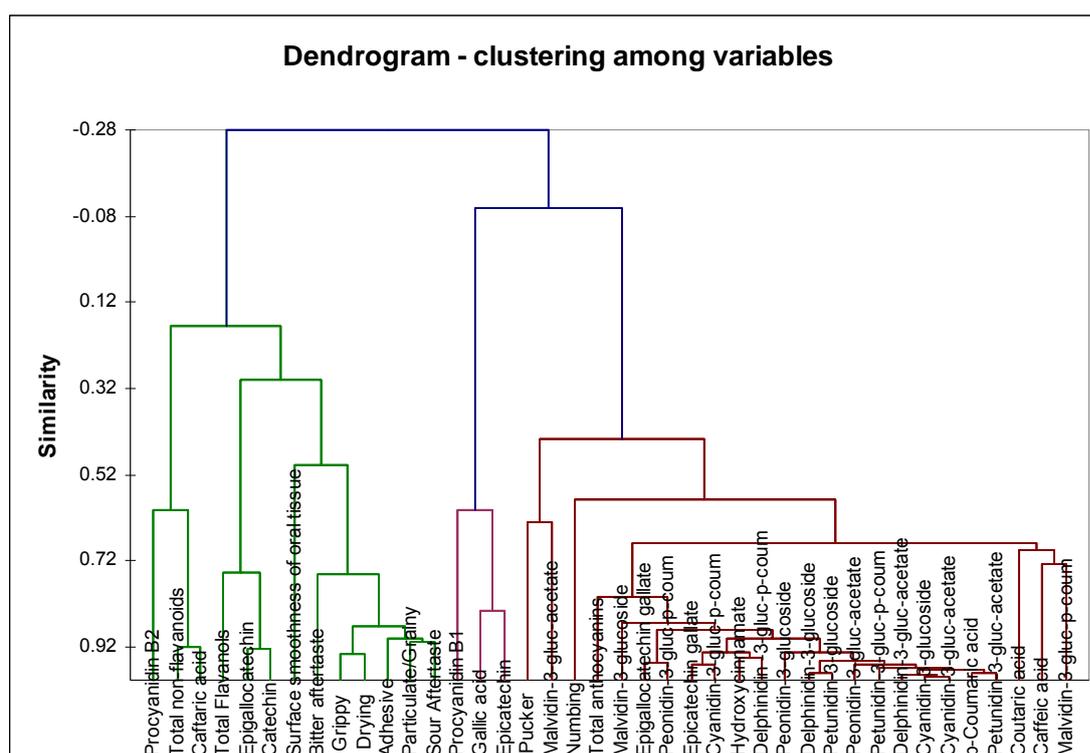
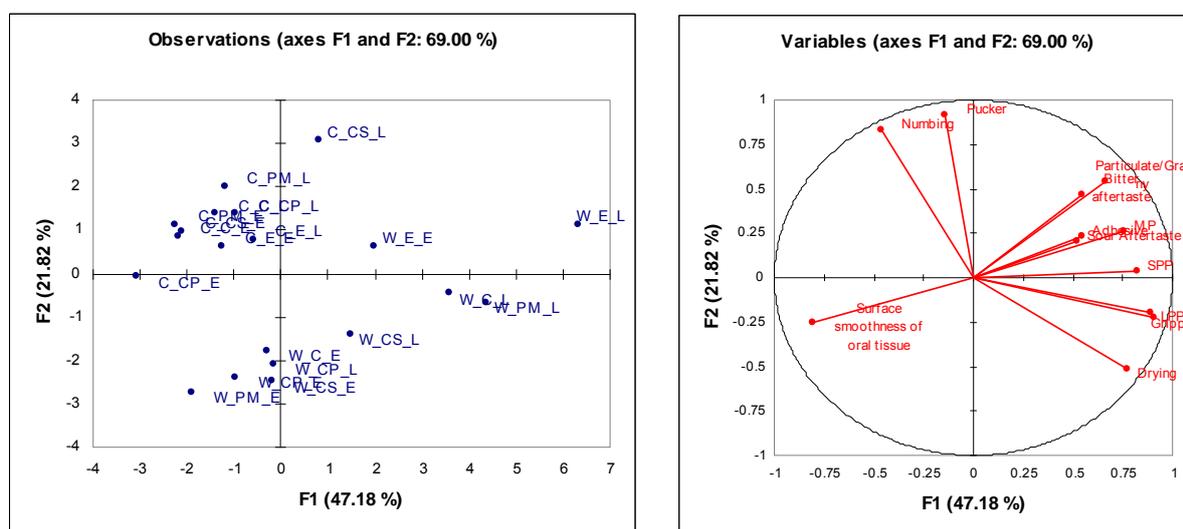


Figure 4.12: Clustering among mouth feel descriptors and phenolic compounds.

#### 4.4.11 RELATIONSHIP OF MP, SPP AND LPP WITH MOUTHFEEL PROPERTIES OF WINE

A PCA was performed to investigate the interrelationships between the monomeric pigments (MP), short polymeric pigments (SPP) and long polymeric pigments (LPP) with mouth feel attributes. LPP, MP and SPP are strongly correlated with each other and with PC1 (Figure 4.13). Furthermore, the compounds are more associated with samples from the warm climatic region, late harvest and specifically with the control, enzyme and cold soak treatments. SPP, LPP and MP are correlated with bitter after taste, particulate, sour aftertaste, grippy and drying mouth feel attributes. Furthermore, MP, SPP and LPP are negatively correlated (Pearson's correlation co-efficient of  $P < 0.05$ ) with procyanidin B1, epicatechin and gallic acid.



**Figure 4.13:** PCA scores and loadings plot showing the relationship of MP, SPP and LPP based on phenolic composition and mouth feel properties of the wine.

#### 4.5 CONCLUSION

Three experimental factors were evaluated in this study, namely climatic region, ripeness level and tannin extraction method. Of these three, climatic region had the biggest effect on mouth feel and phenolic composition.

The wines from the cooler region were generally associated with higher levels of total non-flavonoids and total anthocyanins and more intense numbing and puckering sensations. In contrast, the wines from the warmer region as a group was associated with a more drying and grippy mouth feel as well as less total anthocyanins and total non-flavonoids. There was also evidence that a warmer climate may encourage the

binding of *p*-coumaric acid and delphinidin-3-glucoside, although this must still be confirmed in a follow-up vintage.

Within the group of wines harvested in a cooler climate, the ripeness level had a larger impact on the mouth feel and phenolic composition than the treatment effects. There was a trend that the earlier harvested samples were more adhesive and grippy and had a finer surface smoothness overall, whereas the riper samples were generally more bitter and numbing. In the cooler region, the ripeness level also impacted on the phenolic composition of the wines. The wines that were harvested at a riper stage were associated with many of the anthocyanins/anthocyanin derivatives and were negatively associated with hydroxycinnamate, procyanidin B1 and delphinidin-3-glucoside-*p*-coumaric acid. The inverse relationship between *p*-coumaric acid and delphinidin-3-glucoside-*p*-coumaric acid was observed again, where *p*-coumaric acid was associated with riper grapes. Like with the wines from the cooler region, riper grapes resulted in a coarser surface smoothness, a more numbing sensation, bitter aftertaste and less adhesive mouth feel. In terms of phenolic composition, the riper grapes were again associated with anthocyanins/anthocyanin derivatives, but were this time strongly associated with procyanidin B2, caffeic acid, *p*-coumaric acid, catechin and coumaric acid and total non-flavonoids.

The effect of tannin extraction method on the sensory properties of the wines from the warmer region was more pronounced than in the wines from the cooler region. However, within both regions there was a larger variance between treatments when riper grapes were used, in terms of both mouth feel and phenolic composition. In both regions the specific effect of the treatments on mouth feel changed as the ripeness levels of the grapes increased. This was especially evident in wines from a cooler climate. In addition, the treatment effect on the phenolic composition of the wines was more pronounced in riper grapes.

However, the enzyme treatment was generally associated with a more drying and adhesive character. Interestingly, the enzyme treatment had a larger effect on mouth feel than the phenolic composition suggested, especially in a cooler climate. This provides further evidence that chemical composition can not always be a direct indicator of perceived sensory attributes.

It also appears as if the cold soak treatment generally had the smallest effect on mouth feel and phenolic composition, while the post maceration treatment had the largest effect, regardless of ripeness or region. The control and cold soak treatments were consistently associated with cyanidin-3-glucoside-acetate in grapes harvested at a lower ripeness level, while the post maceration treatment was consistently associated with catechin, gallic acid and total flavanols in riper grapes.

In conclusion, phenolic composition and mouth feel is strongly influenced by climatic region. In warmer climates, the effect of ripeness on mouth feel is smaller than in cooler climates. The effect of the five tannin extraction methods differed depending on climatic region and ripeness level. At this point it is not clear if the specific way in which astringent mouth feel is manifested in wine can be consistently manipulated by tannin extraction methods. SPP, LPP and MP are also correlated with bitter aftertaste, particulate, sour aftertaste, grippy and drying mouth feel attributes.

**Table 4.7:** Sensory attributes of ten wines from a cool wine production area as determined with descriptive profiling

		March					April				
Sample	p-value	C_E	E_E	CS_E	PM_E	CP_E	C_L	E_L	CS_L	PM_L	CP_L
<i>Aroma</i>											
Underripe/Green fruit	<0.0001	11.13 abc <sup>1</sup>	11.53 ab	3.33 bc	2.22 c	14.50 a	14.86 a	6.11 abc	3.57 bc	11.11 abc	8.91 abc
Fresh/Ripe fruit	<0.0001	30.44 a	35.59 a	15.48 bc	24.83 ab	33.61 a	25.61 ab	26.65 ab	3.70 c	25.71 ab	27.39 ab
Overripe/Jammy fruit	0.129	20.58 a	15.89 a	28.24 a	22.46 a	19.70 a	13.04 a	21.24 a	12.98 a	17.46 a	20.74 a
Fresh veg/leafy/herbs	<0.0001	9.04 bcd	18.08 a	6.20 cde	10.94 abcd	15.22 ab	11.94 abc	3.54 de	1.33 e	10.50 abcd	7.35 bcde
Canned Veg	<0.0001	8.17 cd	2.46 d	17.91 bc	10.50 c	2.63 d	8.64 cd	10.08 c	26.72 a	4.06 cd	7.80 cd
Sulphury	<0.0001	0.65 c	1.67 c	17.25 bc	2.83 c	0.65 c	0.22 c	6.39 c	64.77 a	0.81 c	6.87 c
<i>In-mouth sensations</i>											
Particulate/grainy	0.437	36.65 a	36.20 a	37.33 a	35.46 a	34.48 a	35.35 a	37.93 a	39.63 a	39.08 a	38.76 a
Underripe/Green fruit	0.00	11.73 ab	20.70 a	12.78 ab	14.74 ab	22.98 a	15.07 b	14.79 ab	6.24 ab	15.77 ab	12.33 ab
Fresh/Ripe fruit	<0.0001	27.13 ab	32.61 ab	21.04 b	23.74 ab	29.37 ab	23.00 ab	27.46 a	3.41 c	23.96 ab	27.20 ab
Overripe/Jammy fruit	0.031	17.54 ab	8.50 b	19.15 a	17.44 ab	8.00 b	11.04 ab	14.85 ab	13.93 ab	10.31 ab	14.67 ab
Canned Vegetables	<0.0001	4.73 c	3.37 c	15.49 b	8.63 bc	2.57 c	7.29 c	6.31 c	26.00 a	5.23 c	8.80 bc
Fresh veg/leafy/herbs	<0.0001	12.33 abc	15.60 ab	9.24 bcd	11.48 abc	17.72 a	7.80 bcd	7.63 cd	1.31 d	12.88 abc	6.20 cd
Sulphury	<0.0001	0.56 b	0.69 b	2.24 b	1.54 bc	0.44 b	0.30b	1.44 b	58.71 a	0.75 b	2.72 b
<i>Sensations perceived after expectoration</i>											
Surface smoothness	0.008	44.29 ab	43.41 ab	40.31 b	39.11 bc	42.61 ab	42.26 ab	45.00 ab	50.31 a	43.00 ab	46.54 ab
Adhesive	0.024	37.29 abc	39.06 ab	36.78 abc	41.94 a	35.04 bc	33.33 c	33.78 bc	37.17 abc	32.73 c	35.26 abc
Grippy	0.029	16.60 ab	25.09 ab	22.41 ab	20.54 ab	18.09 ab	15.17 b	22.39 ab	26.70 ab	19.52 ab	18.30 ab
Pucker	<0.0001	19.63 ab	19.37 ab	24.89 ab	23.81 ab	16.20 b	22.33 ab	18.98 ab	28.74 a	27.23 ab	15.89 b
Numbing	<0.0001	11.71 b	16.87 ab	19.09 ab	21.44 ab	17.74 ab	23.67 a	22.50 ab	25.54 a	21.83 ab	18.24 ab
Drying	0.001	37.44 cdef	40.50 abc	36.69 def	41.17 ab	37.67 bcdef	38.63 bcde	35.26 ef	42.93 a	34.63 f	39.70abcd
Sour	0.003	34.46 d	36.72 bcd	38.85 abc	41.46 a	34.43 d	40.93 ab	34.78 cd	40.07 ab	36.85 bcd	39.35 ab
Bitter	<0.0001	19.08 e	18.83 de	17.61 cde	20.31 bcde	20.09 bcde	26.72 abc	22.96 bcde	30.65 a	27.33 ab	25.81 abcd

<sup>1</sup>Means with different letters (a, b or c) row-wise are significantly different ( $p \leq 0.05$ )

**Table 4.8:** Sensory attributes of ten wines from a warm production area as determined with descriptive profiling

Sample	p-value	C_E	C_L	CP_E	CP_L	CS_E	CS_L	E_E	E_L	PM_E	PM_L
<i>Aroma</i>											
Ripe/fresh berries	< 0.0001	38.57 ab <sup>1</sup>	40.2 ab	47 a	39.04 ab	29.63 bc	35.33 bcd	22.76 d	22.41 d	37.52 abc	26.93 cd
Jammy	0.026	23.28 c	33.3 abc	27.93 bc	30.11 bc	43.33 a	28.15 bc	33.19 abc	31.67 abc	29.17 bc	37.2 ab
Cordial	< 0.0001	19.35 a	10.22 bcd	19.02 a	10.54 bcd	10.52 bcd	17.39 ab	1.28 e	8.15 cde	11.94 abc	4.43 de
Fresh green	0.011	17.52 a	8.48 bc	7.65 bc	4.76 c	9.28 bc	9.38 bc	7.17 bc	4.96 c	13.11 ab	9.76 bc
Canned veg	< 0.0001	1.61 de	0.7 e	1.72 de	1.52 de	7.37 cd	3.59 de	20.13 a	13.36 bc	1.39 de	13.6 b
Stuffy aroma	< 0.0001	8.65 cd	2.10 d	6.09 cd	14.07 bc	4.24 d	13.02 bc	36.02 a	20.48 b	6.85 cd	14.24 bc
Earthy aroma	0.066	7.67 abcd	11.63 ab	7.02 abcd	9.26 abcd	5.52 cd	12.52 a	9.98 abc	6.72 bcd	3.72 d	7.87 abcd
Savoury veg	0.000	18.54 cd	24.02 abcd	15.59 d	17.48 d	18.67 cd	19.39 bcd	27.78 ab	26.71 abc	21.79 bcd	32.20 a
<i>In mouth sensations</i>											
Particulate/Grainy	< 0.0001	34.72 cde	40.72 abc	33.61 de	34.76 cde	35.15 cde	37.90 bcd	45.35 a	43.70 ab	31.39 e	37.46 bcde
Ripe/fresh berries	< 0.0001	41.49 ab	38.74 abc	45.33 a	41.62 ab	28.83 cd	33.37 bc	32.87 bcd	23.13 d	32.89 bcd	27.63 a
Jammy	0.008	14.61 b	23.80 b	21.65 b	25.96 b	23.85 b	22.93 b	21.93 b	22.41 b	17.39 b	32.28 bcd
Unripe/green fruit	0.571	19.76 a	16.15 a	10.43 a	14.17 a	15.07 a	17.63 a	19.67 a	19.57 a	19.48 a	38.80 a
Fresh green	0.028	19.35 ab	12.04 c	12.67bc	10.90 c	13.74 abc	13.90 abc	16.26 abc	15.37 abc	19.43 a	11.87 a
Savoury veg	0.063	14.28 b	18.54 ab	15.74 b	15.35 b	16.17 b	18.59 ab	15.65 b	25.39a	16.07 b	11.96 c
<i>Sensations perceived after expectoration</i>											
Surface smoothness	< 0.0001	56.76 abc	47.26 de	57.96 abc	63.20 a	51.22 cd	60.13 ab	52.02 bcd	41.96 e	62.63 ab	41.61 e
Adhesive	< 0.0001	37.80 bc	34.99 c	36.35c	33.64 c	33.33 c	35.57 c	43.79 ab	48.91 a	34.83 c	39.61 bc
Grippy	< 0.0001	28.46 bcd	34.89 ab	24.80 cd	22.00 d	30.39 bc	29.78 bc	34.46 ab	41.72 a	24.85 cd	41.78 a
Pucker	< 0.0001	11.30 cd	15.33 abc	8.72 cd	7.72 d	8.63 d	7.30 d	21.39 a	18.48 ab	11.96 bcd	13.65 bcd
Drying	< 0.0001	38.59 bcd	44.61 abc	37.30 cd	34.17 d	40.61 bcd	35.43 d	45.70 ab	45.70 ab	35.98 d	49.85 a
Numbing	0.001	5.78 c	10.48 abc	7.13 c	10.43 bc	7.26 c	13.43 ab	14.26 ab	14.67 ab	7.00 c	15.92 a
Sour Aftertaste	0.000	40.43 bc	40.89 bc	38.78 bc	34.98 c	39.28 bc	39.50 bc	43.63 ab	48.51 a	37.07 bc	34.35 c
Bitter aftertaste	0.019	23.70 ab	24.72 ab	19.04 b	23.45 ab	18.74 b	24.17 ab	22.89 ab	26.39 a	18.37 b	27.63 a

<sup>1</sup>Means with different letters (a, b or c) row-wise are significantly different ( $p \leq 0.05$ )

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# **Chapter 5**

## **General discussion and conclusions**

## 5. GENERAL DISCUSSION AND CONCLUSIONS

### 5.1 DISCUSSION AND CONCLUSION

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From the inception of winemaking (McGovern, 2003) to the modern age of winemaking, the winemaker always strives to obtain the best quality from grape berries as possible. One objective is to discover the optimal method for tannin and anthocyanin extraction from grapes. For example, Pinot noir, also known as the heart-break grape, is notorious for its poor anthocyanin extraction; additional measures must be taken to extract sufficient anthocyanins from this grape variety (Scollary, 2010). Then, in the mid 1980's, a Lebanese winemaker called Guy Accad (Norman, 2010) had the revolutionary idea to leave the skins on the juice for a couple of days at very low temperature prior to fermentation. And so the idea of cold maceration was born. The influence of phenolic ripeness is also very important in the extraction of anthocyanins and tannins. Glories developed in 1984 a method to determine the phenolic ripeness of grape berries (Glories, 1984).

The specific aims of this study were to evaluate the phenolic ripeness of the grapes with the Glories method and to evaluate the extraction of tannin and anthocyanin by the winemaking processes of cold maceration, post maceration, a combination of cold and post maceration, and the use of pectolytic enzymes. Further objectives were to evaluate the extraction of tannin concentration by using two precipitation methods and to evaluate the effect of the different winemaking processes on the mouth feel of the wine.

It is sometimes feared that leaving grapes to reach optimal phenolic ripeness will result in wines with a high alcoholic content. However, in 2008 it was found in this study that Shiraz grapes from Plaisir de Merle was phenolic ripe at a sugar level of 24°B and that a Cabernet Sauvignon from Morgenster at 23.5°B. This clearly shows that at low traditional ripeness levels some grapes may have already achieved phenolic ripeness. Repeating this study in 2009 and 2010 gave different and unexpected results.

There are different tannin analysis methods, but the most preferred methods are the tannin precipitation methods of methyl cellulose (MCP) and bovine serum albumin (BSA) method. The BSA method was developed in California by Hagerman and Butler (1978) and was

later modified by Harbertson (2003) to include a bisulfite bleaching process. The BSA method is also a very time-consuming method. The MCP method was developed in Australia by Sarneckis *et al.* (2006) and is a much quicker method for the analysis of total tannin. These two methods gave more or less the same results, although the BSA method's results are much lower in values. The BSA method makes it difficult to follow the tannin concentration of grapes as grapes do not release tannins that easily. Also, the fact that there are a lot of proteins present in the grape juice can cause interference with one of the processes of the BSA method. There are some different views on these methods. It was found that the correlation between the BSA and MCP method was very good ( $R^2 = 0.88$ ), but the correlations between the BSA ( $R^2 = 0.28$ ) and MCP ( $R^2 = 0.32$ ) and the HPLC was very poor. The reason for the poor correlation between the BSA and MCP methods and the HPLC method is because the standards for the HPLC did not include polymeric tannins and that the HPLC was standardized with only monomers and dimers (B1 & B2). Sarneckis *et al.* (2006) found that there was a good correlation between the MCP method and reverse phase-HPLC, but Seddon and Downey (2008) found that there was no correlation between the MCP and BSA methods and reverse phase-HPLC. This was confirmed in studies done by Brooks *et al.* (2008), Harbertson *et al.* (2008) and Harbertson and Downey (2009) which found that the BSA methods were invalid as there was no repeatability and accuracy.

The study on tannin concentration showed that the grapes from the warmer climate resulted in higher amount of tannin concentration than cooler climates. Further results showed that the cooler area were associated with higher total non-flavonoids and total anthocyanins, while the warmer area were associated with lower concentration of total non-flavonoids and total anthocyanins. In the 2008 harvesting season the treatment of CM showed an effect in tannin extraction, but this was not repeated in the 2009 harvesting season. The sensory study showed that the CM treatment had a small effect on mouth feel and phenolic composition. In 2009 the PM and the combination treatment of CM+PM had an effect on tannin concentration. This was due to the fact that the skins and pips had a longer contact with the wine and more tannin was extracted. PM also had the largest effect on mouth feel as it was associated with catechin, gallic acid and total flavonoids. In 2009 the enzyme treatment had the biggest effect on tannin extraction. The enzyme treatment had the largest effect on mouth feel and associated with a drying and adhesive mouth feel

sensation. This shows that using pectolytic enzymes during the first stages of the winemaking process, more tannin can be released from the cell wall components. In some instances the enzyme treatment had such an effect that it overshadowed the effect of the winemaking processes of cold and post maceration. The results showed that when the grapes have not fully reached phenolic ripeness treatments like CM and CM+PM will extract more tannin. As soon as the grapes are phenolic ripe only the enzyme treatment had an effect on tannin concentration.

In both harvesting seasons (2008 & 2009) the amount of total anthocyanin was higher in the cooler climate. This confirms the challenge with which viticulturists are faced to get a balance between tannin and anthocyanin. Also in both the harvesting years the treatment of CM had no real effect on anthocyanin extraction. Patterson (June, 2009) found that there was two schools of thought on cold maceration. One theory states that cold maceration has no effect and that the grapes will elute the anthocyanin that it has, although cultivar will play a part. The other theory is that cold maceration extracts more colour than normal winemaking practices. In this study it was found that cold maceration has no real effect on total anthocyanin. In 2008 Cabernet Sauvignon from Plaisir de Merle had lower total anthocyanins than Shiraz, while on Morgenster the Cabernet Sauvignon had higher total anthocyanin than the Shiraz. This was due to Botrytis rot that was in the Shiraz that year. In 2009 Cabernet Sauvignon had higher total anthocyanin than Shiraz from Plaisir de Merle and on Morgenster the Cabernet Sauvignon had lower total anthocyanin than Shiraz. Factors like climate/terroir together with viticultural practices will play a role in the difference of the total anthocyanins.

Colour density shows a good correlation with total anthocyanins as colour density follow the same tendency. It was with the use of the enzyme treatment that the colour density increased dramatically and will probably have a more stabilizing effect in the long run. Hue showed that young wines will have low values and these values will increase as the wine matured. This was due to different factors like oxidation, maturation or even enzymatic oxidation as a result of an excess of laccase. Otherwise none of the treatments had a negative effect on hue.

The MP was high in the wine directly after AF, but decreases after BOT. This happened as the monomers polymerized with each other to form polymers. This resulted in an increase in SPP from AF to BOT. The LPP was formed after AF in the bottle as the concentration of the LPP was very low at bottling and started to increase as the wine matures. These SPP, LPP and MP are also correlated with bitter after taste, particulate, sour aftertaste, grippy and drying mouth feel attributes.

## 5.2 FINAL CONCLUSION

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In evaluating the two different precipitation methods, it was found that the MCP method is the best and quickest method for analysis of total tannin concentration. If the evolution of monomeric, small and large polymer pigments are to be followed the BSA method is then the best although more difficult and much longer to do. In 2008 the Glories method for phenolic ripeness shows interpretable results, but in 2009 the results was much more difficult to interpret. Further research is therefore needed to understand the phenolic ripeness of different cultivars in different terroirs. The different winemaking techniques showed variable results. Some treatments had an effect in one year but a different effect in the next year. The enzyme treatment had the highest concentration of tannin as the pectolytic activity of the enzymes brakes the bonds between the tannin and the cell wall components. The enzyme treatment was generally associated with a more drying and adhesive character. The wines from the cooler region were generally associated with higher levels of total non-flavonoids and total anthocyanins and more intense numbing and puckering sensations. In contrast, the wines from the warmer region as a group was associated with a more drying and grippy mouth feel as well as less total anthocyanins and total non-flavonoids. There was also evidence that a warmer climate may encourage the binding of *p*-coumaric acid and delphinidin-3-glucoside, although this must still be confirmed in a follow-up vintage. There was a trend that the earlier harvested samples were more adhesive and grippy and had a finer surface smoothness overall, whereas the riper samples were generally more bitter and numbing.

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**ADDENDUM A: Chemical analysis of the grapes harvested in**

		<b>Must - 2008</b>			
<b>Cultivar</b>	<b>Farm</b>	<b>°B</b>	<b>pH</b>	<b>TS</b>	<b>K</b>
Cab Sauv	PdM	22.7	2.51	5.98	1908
Cab Sauv	PdM	27.1	3.69	5.10	2626
Shiraz	PdM	23.7	3.63	4.81	1980
Shiraz	PdM	27.0	4.00	2.88	2440
Cab Sauv	Morg	20.5	3.27	11.04	1960
Cab Sauv	Morg	25.0	3.60	3.80	1530
Shiraz	Morg	23.6	3.89	6.98	1710
Shiraz	Morg	24.7	3.44	8.48	1920

		<b>Must - 2009</b>			
<b>Cultivar</b>	<b>Farm</b>	<b>°B</b>	<b>pH</b>	<b>TS</b>	<b>K</b>
Cab Sauv	PdM	20.9	3.10	11.50	1320
Cab Sauv	PdM	23.8	3.32	6.13	1300
Shiraz	PdM	23.4	3.46	6.31	1950
Shiraz	PdM	24.8	3.60	5.17	2020
Cab Sauv	Morg	21.1	3.22	8.41	1100
Cab Sauv	Morg	23.0	3.34	6.05	1260
Shiraz	Morg	23.9	3.62	4.08	1440
Shiraz	Morg	23.9	3.62	4.08	1260

**ADDENDUM B: Chemical analysis of the wines harvested in 2009**

Cultivar	Farm	Wine nr.	°B	Treatm	FSO2	TSO2	pH	TA	Alc	RS	VA	Malic	Lactic
Cab Sauv	PdM	60207	20.9	C	19	53	2.95	9.72	11.46	0.93	0.41	3.43	-0.13
		60208		E	15	44	2.88	10.04	11.69	1.30	0.42	3.66	-0.18
		60209		CM	14	48	2.94	9.64	11.83	1.33	0.41	3.26	0.12
		60210		PM	14	70	2.94	9.83	11.58	1.17	0.42	3.51	-0.12
		60211		CM+PM	11	63	2.99	9.49	10.75	1.17	0.44	3.24	0.07
		20301	23.8	C	22	62	3.30	8.58	14.69	0.67	0.38	2.60	-0.03
		20302		E	17	44	3.20	8.60	14.90	1.87	0.41	2.69	-0.12
		20303		CM	24	64	3.20	9.21	14.74	0.70	0.32	2.92	-0.02
		20304		PM	11	39	3.27	8.61	15.10	0.60	0.35	2.64	-0.07
		20305		CM+PM	27	56	3.22	8.72	15.56	1.57	0.43	2.43	-0.07
Shiraz	PdM	180208	23.4	C	19	57	3.20	8.04	13.48	0.90	0.33	2.93	-0.03
		180209		E	22	63	3.17	8.18	13.57	1.33	0.40	2.77	-0.12
		180210		CM	21	67	3.21	7.68	13.59	1.13	0.38	2.40	-0.05
		180211		PM	22	67	3.23	7.91	13.58	0.83	0.40	2.65	-0.02
		180212		CM+PM	13	48	3.22	7.66	13.47	1.00	0.36	2.34	0.04
		20311	24.8	C	19	62	3.31	7.87	14.57	1.23	0.36	2.62	0.01
		20312		E	21	67	3.31	7.63	14.69	1.77	0.44	2.41	0.00
		20313		CM	19	63	3.36	7.68	14.62	1.00	0.42	2.57	-0.06
		20314		PM	19	52	3.33	7.73	14.63	1.33	0.41	2.53	-0.02
		20315		CM+PM	22	65	3.32	7.15	13.64	1.07	0.43	1.74	0.04

Cultivar	Farm	Wine nr.	°B	Treatm	FSO2	TSO2	pH	TA	Alc	RS	VA	Malic	Lactic
Cab Sauv	Morgenster	120318	21.1	C	25	68	3.28	8.47	11.98	0.83	0.32	3.28	0.10
		120319		E	25	66	3.22	8.77	12.01	1.40	0.37	3.38	0.03
		120320		CM	29	70	3.40	7.67	12.11	0.73	0.38	2.69	0.25
		120321		PM	14	65	3.35	8.49	12.12	0.60	0.56	3.27	0.07
		12032		CM+PM	12	56	3.50	6.72	12.27	1.17	0.60	0.78	1.24
		140401	23	C	22	66	3.50	7.98	13.95	1.20	0.33	2.95	-0.02
		140402		E	19	60	3.38	8.62	13.93	1.90	0.33	3.17	-0.12
		140403		CM	20	54	3.48	8.02	14.18	1.87	0.33	2.88	-0.10
		140404		PM	20	57	3.48	8.09	14.15	1.00	0.36	2.87	0.10
		140405		CM+PM	14	41	3.53	7.52	14.18	1.70	0.36	2.80	-0.14
Shiraz	Morgenster	240303	21.8	C	20	57	3.21	6.89	11.81	1.50	0.28	2.12	0.11
		240304		E	11	50	3.17	6.98	11.85	2.05	0.32	2.06	0.04
		240305		CM	21	62	3.24	6.87	11.88	1.23	0.31	1.98	0.21
		240306		PM	28	63	3.33	5.87	11.87	1.43	0.44	1.22	0.37
		240307		CM+PM	26	60	3.39	5.80	12.11	1.10	0.45	0.77	0.79
		140406	23.9	C	40	79	3.51	6.64	14.51	1.37	0.38	1.85	-0.08
		140407		E	29	76	3.45	7.07	14.33	2.13	0.35	2.05	0.04
		140408		CM	26	69	3.53	6.41	14.60	1.47	0.33	1.78	0.03
		140409		PM	30	69	3.50	6.71	14.68	1.50	0.37	1.95	0.08
		140410		CM+PM	24	55	3.51	6.40	14.70	1.70	0.37	1.88	0.00

**ADDENDUM C: Aroma and flavour recognition guide – Phase 1**

**Fruity Flavours**

Sour and/or slight green note  
associated with fruits not yet  
ready for eating

**UNRIPE FRUITS**

Cooked, syrupy,  
viscous

**JAMMY**

Fresh, tart, lively

**RED BERRIES**

**Green Flavours**

Sharp vegetative notes  
associated with grass, fresh  
herbs and green stalks

**FRESH VEGETATIVE**

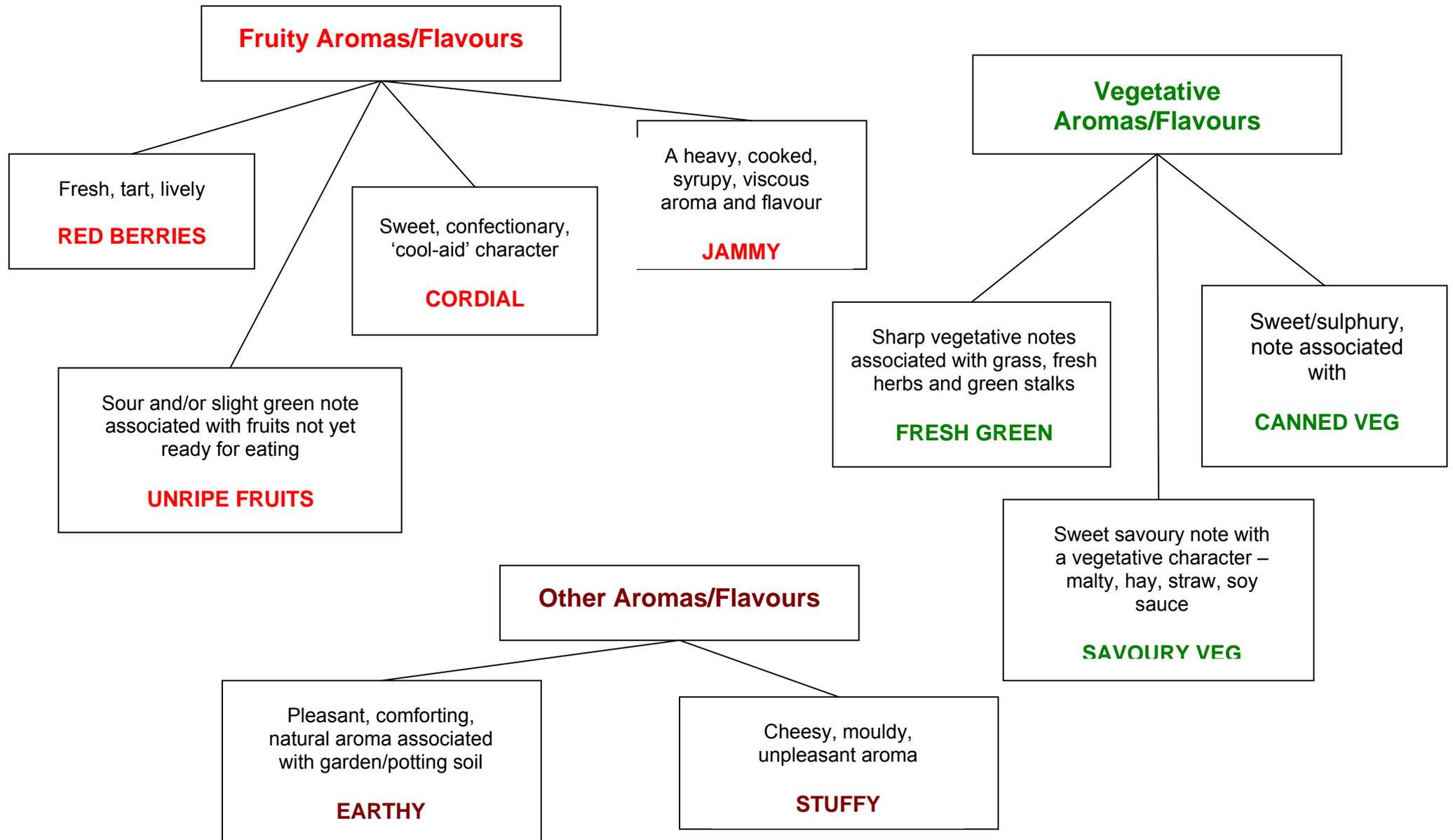
Reminiscent of the  
smell of a  
mushroom farm

**SULPHURY**

Slightly sulphury,  
stuffy note  
associated with

**CANNED VEG**

**ADDENDUM C: Aroma and flavour recognition guide – Phase 2**



## ADDENDUM D: Mouth feel evaluation guide – Phase 1 and Phase 2

